Crafts) and with phenyl-, *m*-xylyl- and mesitylmagnesium bromides are summarized. The condensation with the 1-carbonyl group in the anhydride increases as the number of methyl groups ortho to the new point of attachment increases. This effect is considerably more noticeable in the Grignard reactions than in the Friedel–Crafts.

COLUMBUS, OHIO RECEIVED JUNE 9, 1950

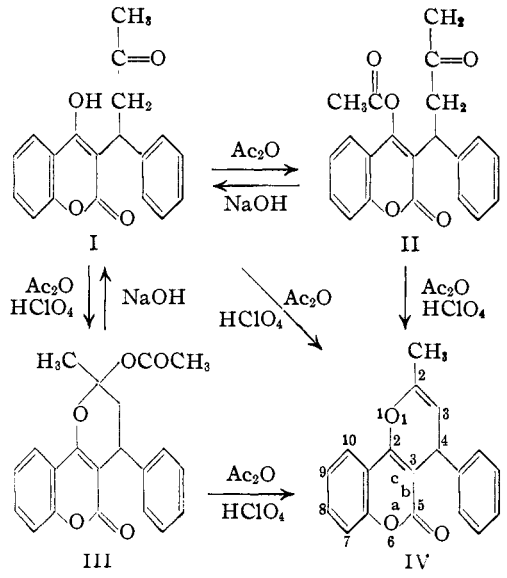
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Studies on 4-Hydroxycoumarins. X. Acylation of 3-(α-Phenyl-β-acetylethyl)-4-hydroxycoumarin[1]

BY MARTIN SEIDMAN, DALE N. ROBERTSON AND KARL PAUL LINK

Shortly after the anticoagulant 3,3′-methylene-bis-(4-hydroxycoumarin), (Dicumarol®), was isolated from spoiled sweet clover hay,[2] identified and synthesized,[3,4] its marked toxicity in the rat was noted.[5] It was also indicated[6] that many other 3-substituted-4-hydroxycoumarins possess powerful *in vivo* anticoagulant activity in many animal species and a complete account of their relative activity in the rabbit has been published.[7] Recently, O'Connor reported the successful use of Dicumarol® as a rodenticide.[8] It was felt desirable therefore to restudy the more potent compounds in the rat especially for rodenticidal potentialities. The results of this survey[9] revealed that 3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin (I) is approximately fifty times more lethal and in addition the time to effect kill is about half that required by Dicumarol®. A study of the reactions of I was consequently undertaken. This paper reports an improved synthesis and various ester derivatives.

I is prepared by a Michael addition of 4-hydroxycoumarin to benzalacetone.[10] By refluxing the reactants in dioxane containing piperidine as catalyst for four hours a yield of 67% of pure product has been realized. Acetylation of I under different conditions has produced three derivatives. When I is refluxed with acetic anhydride the acetate II, m. p. 117–118°, is obtained. If, however, I is treated with acetic anhydride containing perchloric acid as catalyst the acetate III, m. p. 204–205°, can be isolated in small yield. By allowing this reaction to proceed for five minutes the dehydrated product 2 - methyl - 4 - phenyl - 5 - oxo - γ - pyrano - (3,2-c)(1)-benzopyran (IV) crystallizes out. This dehydration parallels that of 4-salicylbutan-2-one to form 2-methylchromene.[11]



The assignment of the structures II and III is based upon the following considerations:

1. The compound with the higher melting point is assumed to be the more symmetrical and have the more rings.

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. This work is from the thesis submitted by Martin Seidman to the faculty of the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy, July, 1950. This paper was presented before the Division of Organic Chemistry at the 117th Meeting of the American Chemical Society, Philadelphia, April, 1950. Subsequent to the submission of this paper, the coined name "warfarin" was assigned to the Anticoagulant Rodenticide 3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin, S. A. Rohwer, U. S. Dept. Agr. Interdepartmental Comm. Pest Control, June 29, 1950. The chemical name 3-(α-acetonylbenzyl)-4-hydroxycoumarin is the systematic name selected by *Chemical Abstracts* for warfarin.

(2) H. A. Campbell and K. P. Link, *J. Biol. Chem.*, **138**, 21 (1941).

(3) M. A. Stahmann, C. F. Huebner and K. P. Link, *ibid.*, **138**, 513 (1941).

(4) K. P. Link, *Harvey Lecture Series*, **39**, 162 (1943–1944).

(5) R. S. Overman, J. B. Field, C. A. Baumann and K. P. Link, *J. Nutrition*, **23**, 589 (1942).

(6) R. S. Overman, M. A. Stahmann, W. R. Sullivan, C. F. Huebner, H. A. Campbell and K. P. Link, *J. Biol. Chem.*, **142**, 941 (1942).

(7) R. S. Overman, M. A. Stahmann, C. F. Huebner, W. R. Sullivan, L. Spero, D. G. Doherty, M. Ikawa, L. Graf, S. Roseman and K. P. Link, *ibid.*, **153**, 5 (1944).

(8) J. A. O'Connor, *Research*, **1**, 334 (1948).

(9) By Dr. Lester D. Scheel and Mrs Dorothy W. Ballou. A complete account of this survey is in the Masters thesis of Dorothy L. Wu, Biochemistry Department, University of Wisconsin, 1949. Recent unpublished findings in this laboratory have shown that less than a total of 1.0 mg. of 3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin administered daily will kill the average 250-g. laboratory rat in five to ten days.

(10) M. Ikawa, M. A. Stahmann and K. P. Link, THIS JOURNAL, **66**, 902 (1944).

(11) W. Baker and J. Walker, *J. Chem. Soc.*, 646 (1935).

2. III can be converted to IV by shaking with acetic anhydride containing perchloric acid for five minutes at room temperature. When II is treated similarly it can be recovered unchanged. II can be made to lose acetic acid however, to give IV by heating in acetic anhydride–perchloric acid solution at 50° for fifteen minutes.

3. The infrared spectra[12] of II and III as mulls in mineral oil exhibited absorption bands corresponding to the ester C=O at 1755 cm.$^{-1}$ and 1728 cm.,$^{-1}$ respectively. These findings are in agreement with the observation that the ester C=O bands in phenyl acetate and vinyl acetate appear at frequencies about 30 cm.$^{-1}$ higher than in unconjugated esters.[13]

Both acetates regenerate I after alkaline hydrolysis.

Attempts to acylate I by refluxing it with propionic anhydride or chloroacetyl chloride or by treatment with propionic anhydride containing perchloric acid resulted in the formation of IV. The propionate ester has been made by reaction of I with propionic anhydride in pyridine for six days. In a similar manner using the corresponding acid chlorides the benzoate and *p*-nitrobenzoate esters have been prepared.

Experimental

Preparation of 3-(α-Phenyl-β-acetylethyl)-4-hydroxycoumarin (I).—The method is a variation of the procedure developed by Ikawa, Stahmann and Link.[10] Eleven grams of 4-hydroxycoumarin and 10 g. of benzalacetone were refluxed for four hours in 40 ml. of dioxane containing 1 ml. of piperidine. The solution was then cooled and poured into 1 l. of ice and water with vigorous stirring. An oil separated which gradually crystallized. The solid was filtered and recrystallized from acetone–water, yield 14.1 g. (67%), m. p. 161°.

3-(α-Phenyl-β-acetylethyl)-4-hydroxycoumarin Acetate (II).—Twenty grams of I was refluxed with 150 ml. of acetic anhydride for one hour. After cooling, the solution was poured into 1 l. of ice and water with vigorous stirring. An oil separated which turned to a gum and then a solid. After filtration and recrystallization from methanol 16.5 g. of II was obtained, m. p. 117–118°.

Anal. Calcd. for $C_{21}H_{18}O_5$: C, 72.00; H, 5.14. Found: C, 72.25; H, 5.31.

Preparation of 2-Methyl-4-phenyl-5-oxo-γ-pyrano(3,2-c)(1)benzopyran (IV).—This compound was prepared from I by the following procedures:

(a) Reaction with Acetic Anhydride and Perchloric Acid.—Four grams of I was suspended in 25 ml. of acetic anhydride and two drops of perchloric acid was added. Upon shaking, the solid gradually dissolved. After five minutes a white crystalline material separated. This was immediately filtered and washed with glacial acetic acid. The compound melted at 145–146°, yield 2 g. By pouring the mother liquor into ice water an additional 1.4 g. was obtained. The product was recrystallized from methanol for analysis.

Anal. Calcd. for $C_{19}H_{14}O_3$: C, 78.62; H, 4.83. Found: C, 78.80; H, 4.99.

(b) Reaction with Propionic Anhydride.—Five grams of I was refluxed with 40 ml. of propionic anhydride for one hour. The solution was cooled and poured into 500 ml. of ice and water. An oil separated. The water was decanted and 50 ml. of methanol was added, whereupon the oil crystallized. After two recrystallizations from methanol 2.2 g. of IV, m. p. 145–146°, was obtained.

(c) Reaction with Propionic Anhydride and Perchloric Acid.—Four grams of I was suspended in 30 ml. of propionic anhydride containing two drops of perchloric acid. After fifteen minutes the clear yellow solution was poured into 500 ml. of ice and water with stirring. The oil which separated was treated with 50 ml. of methanol whereupon crystals of IV were obtained; yield 2.8 g.; m. p. 145–146°.

(d) Reaction with Chloroacetyl Chloride.—Four grams of I was refluxed with 40 ml. of chloroacetyl chloride for one hour. After cooling the solution was poured into 500 ml. of ice and water. An oil formed which was separated from the water layer and crystallized by the addition of 50 ml. of methanol. After two recrystallizations from methanol 1.4 g. of IV, m. p. 145–146°, was obtained.

2-Methyl-2-acetoxy-4-phenyl-5-oxo-dihydropyrano-(3,2-c)(1)benzopyran (III).—Five grams of I suspended in 60 ml. of acetic anhydride and two drops of perchloric acid added. After shaking for three minutes almost all of the solid had dissolved. The mixture was then immediately poured into 500 ml. of ice and water with stirring. A gum formed which gradually solidified. The precipitate was filtered and recrystallized from isopropyl alcohol; yield 0.9 g., m. p. 185–195°. Two recrystallizations from methanol raised the melting point to 204–205°.

Anal. Calcd. for $C_{21}H_{18}O_5$: C, 72.00; H, 5.14. Found: C, 71.96; H, 5.24.

From the mother liquor a small amount of pure IV was obtained.

Conversion of II to IV.—Two grams of II was suspended in 15 ml. of acetic anhydride and three drops of perchloric acid added. The mixture was heated to 50° on a steam-bath and a dark green solution resulted. After fifteen minutes the solution was cooled and poured into 500 ml. of ice and water with stirring. A gum formed which crystallized upon standing. The precipitate was filtered and recrystallized from methanol; yield 0.7 g., m. p. 145–146°. The melting point when mixed with pure IV was 145–146°.

Conversion of III to IV.—Two hundred milligrams of III was added to 20 ml. of acetic anhydride and two drops of perchloric acid added. After five minutes a clear solution resulted. This was poured into 500 ml. of ice and water to give a white precipitate. After recrystallization from ethanol–water 125 mg. of IV was obtained, m. p. 144–146°.

Hydrolysis of II to I.—Six hundred milligrams of II was heated with 50 ml. of 20% sodium hydroxide solution for four hours on a steam-bath. A red oil separated. The mixture was diluted with 200 ml. of water and acidified with concentrated hydrochloric acid. A white precipitate formed, yield 0.4 g. After recrystallization from acetone–water the melting point was 158–160°.

Hydrolysis of III to I.—Two hundred milligrams of III was refluxed with 50 ml. of 95% ethanol and 50 ml. of 10% sodium hydroxide solution for three hours. The resulting solution was diluted with 200 ml. of water and acidified with concentrated hydrochloric acid. The white precipitate which formed was recrystallized from acetone–water. The 150 mg. thus obtained melted at 151–157°. Another recrystallization raised the melting point to 157–158°; the melting point of a mixture of this product with I was 157–161°.

3-(α-Phenyl-β-acetylethyl)-4-hydroxycoumarin Propionate.—A mixture of 3.1 g. of I, 3 ml. of propionic anhydride and 25 ml. of dry pyridine was allowed to stand for six days at room temperature. The solution was then poured into 500 ml. of ice and water with stirring. The gum which formed was dissolved in 40 ml. of hot methanol. Upon cooling 0.6 g. of the desired product crystallized out. After two recrystallizations from methanol the m. p. was 135–137°.

(12) The absorption studies were carried out by K. S. McCallum of the Analytical Chemistry Department of the University of Wisconsin. A full discussion of the results including the experimental data may be found in his Ph.D. thesis submitted to the Graduate School of the University of Wisconsin, 1950.

(13) R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1073 (1949).

Anal. Calcd. for $C_{22}H_{20}O_5$: C, 72.53; H, 5.49. Found: C, 72.33; H, 5.75.

3-(α-Phenyl-β-acetylethyl)-4-hydroxycoumarin Benzoate.—To a solution of 10 g. of I in 40 ml. of dry pyridine was added 5.8 g. of benzoyl chloride at room temperature. Crystals formed immediately. After three hours the mixture was poured into 500 ml. of ice and water with stirring. The resulting solid was filtered and after four recrystallizations from acetone–water 4.4 g. of the desired product, m. p. 117–119°, was obtained.

Anal. Calcd. for $C_{26}H_{20}O_5$: C, 75.71; H, 4.89. Found: C, 75.41; H, 5.03.

The **p-nitrobenzoate** of I was prepared in a similar manner; yield 32%; m. p. 194–196°.

Anal. Calcd. for $C_{26}H_{19}O_7N$: C, 68.26; H, 4.19. Found: C, 68.05; H, 4.28.

Summary

1. Acetylation of the new anticoagulant rodenticide 3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin has yielded 3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin acetate, 2-methyl-2-acetoxy-4-phenyl-5-oxo-dihydropyrano(3,2-c)(1)benzopyran and 2-methyl-4-phenyl-5-oxo-γ-pyrano(3,2-c)(1)benzopyran.

2. The propionate, benzoate and *p*-nitrobenzoate esters have been prepared.

MADISON 6, WIS. RECEIVED MAY 25, 1950

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF MARYLAND]

Synthesis of Some Isoquinoline Derivatives Related to Podophyllotoxin

BY WILKINS REEVE AND WILLIAM M. EARECKSON, III[1]

A series of di- and tetrahydro-substituted isoquinolines, having certain of the structural features of podophyllotoxin, has been prepared in the hope some members might also possess the tumor-damaging properties of podophyllotoxin.

The amide (I) was prepared in 81% yield by treating homopiperonylamine with trimethoxybenzoyl chloride in benzene solution in the presence of calcium oxide. Under the usual Schotten–Baumann conditions, the amide is obtained in 65–70% yield. The cyclization of the amide to dihydroisoquinoline (II) was accomplished by the well-known Bischler–Napieralski reaction. Methylation of the dihydroisoquinoline (II) gave its methiodide (III), which on treatment with alkali was converted into a pseudo base. The structure assigned to this compound follows from the neutral character of the compound, its analysis, and the existence of a corresponding non-ionic cyanide (V) prepared from III by treatment with a sodium cyanide solution.

The tetrahydroisoquinoline (VI), prepared by the hydrogenation of II over Adams platinum oxide catalyst, could be methylated with methyl iodide to a mixture consisting of the hydroiodide of the starting material and VIII. However, the monomethyl derivative (VII) could be prepared in practically quantitative yield by the procedure of Clarke, *et al.*,[2] involving the refluxing of VI with formaldehyde and formic acid. Methylation of this with methyl iodide gave the pure quaternary salt (VIII).

The oxidation of all of the di- and tetrahydroisoquinoline compounds was studied in the hope a practical synthesis could be developed for 3′, 4′, 5′-trimethoxy-4,5-methylenedioxybenzophenone-2-carboxylic acid (IX), a compound which would be a valuable intermediate in the synthesis of podophyllotoxin. This compound has previously been obtained in small amounts by the oxidation of podophyllotoxin[3] and by the oxidation of a substituted phenyldihydronaphthalene.[4] Oxidation of the dihydroisoquinoline (II) with potassium permanganate in *t*-butyl alcohol–water mixture gave some of the keto acid (IX) but the main product was the completely aromatized isoquinoline (X). Oxidation of the latter gave negative results. The keto acid (IX) could also be obtained in small yield by the oxidation of compounds IV, VI and VII. Compound VIII was converted to the quaternary hydroxide which easily decomposed to an unsaturated compound. This, however, changed easily to a glassy product and oxidation was unsuccessful.

Experimental

All melting points are corrected. Analyses are by Mrs. Mary Aldridge and Mr. Byron Baer of this Laboratory.

N-(3,4,5-Trimethoxybenzoyl)-homopiperonylamine (I).—This was prepared by the method of Slotta and Haberland[5] by mixing 102 g. (0.616 mole) of homopiperonylamine[6] dissolved in 600 ml. of dry benzene with 142 g. (0.616 mole) of trimethylgalloyl chloride[7] also dissolved in 600 ml. of dry benzene. The thick pasty mass was stirred mechanically, and 55 g. of calcium oxide, ground to pass a 150-mesh sieve, was added over a 90-minute period. The temperature was maintained at 75–80°. The reaction mixture was filtered hot, the filtrate cooled, the amide (53 g., m. p. 133.5–135.5°) filtered off, and the filtrate concentrated to 100 ml. to yield another 18.5 g. of amide. The insoluble material from the reaction mixture was ex-

(1) du Pont Fellow in Chemistry, 1949–1950.

(2) H. T. Clarke, H. B. Gillespie and S. Z. Weishaus, THIS JOURNAL, **55**, 4576 (1933).

(3) E. Spath, F. Wessely and E. Nadler, *Ber.*, **66**, 125 (1933).

(4) R. D. Haworth and T. Richardson, *J. Chem. Soc.*, 348 (1936). The preparation of this keto acid has also been briefly described by W. J. Gensler and C. M. Samour in a Communication to the Editor (THIS JOURNAL, **72**, 3318 (1950), submitted May 27, 1950) in which they claim priority on the synthesis of this compound. They apparently were unaware of the successful synthesis of the keto acid and its methyl ester by R. D. Haworth and T. Richardson in 1936.

(5) K. H. Slotta and G. Haberland, *Angew. Chem.*, **46**, 766 (1933).

(6) W. Reeve and W. M. Eareckson, THIS JOURNAL, **72**, 3299 (1950).

(7) M. Asano and K. Yamaguti, *J. Pharm. Soc. Japan*, **60**, 105 (1940).