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

Studies on 4-Hydroxycoumarins. XV. Synthesis of Some 3-Thio-4-hydroxycoumarins from 3-Bromo-4-hydroxycoumarin[1]

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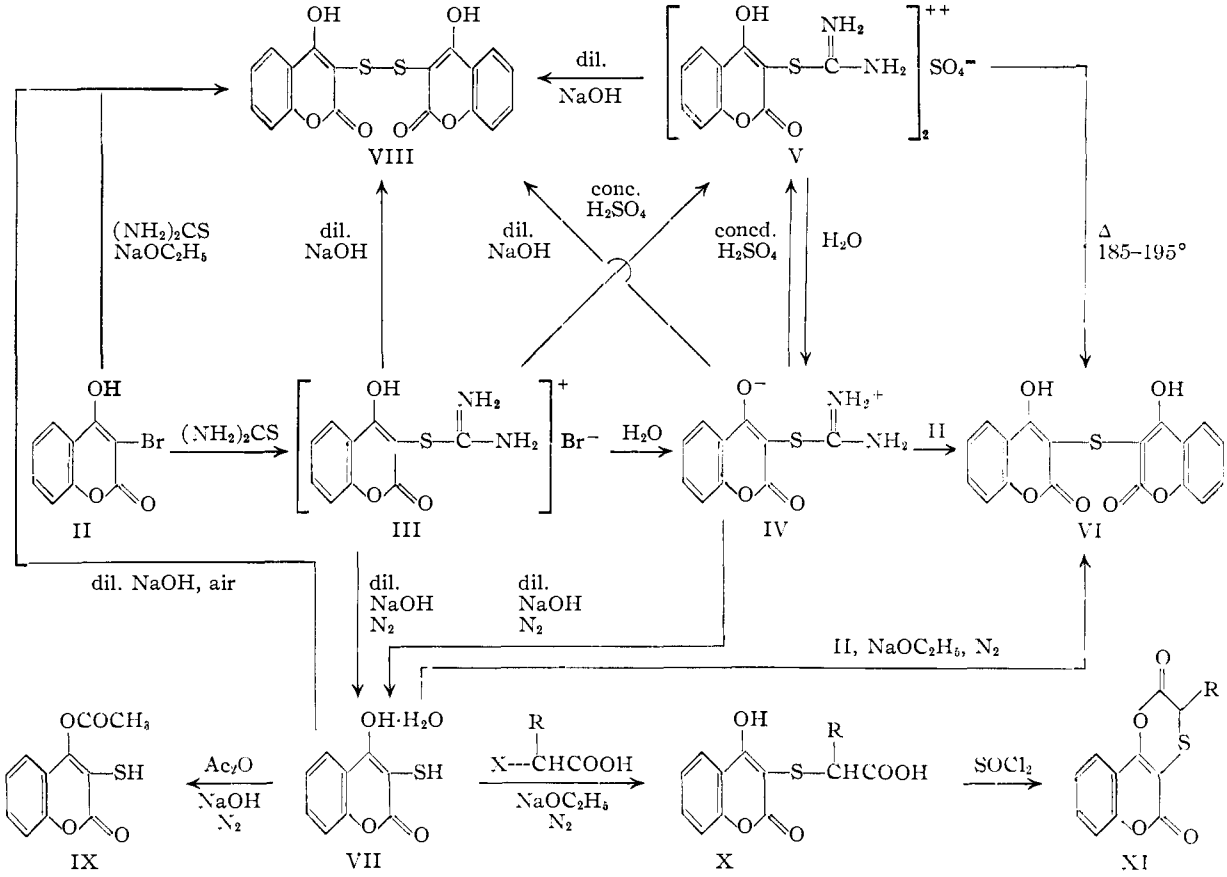
RECEIVED APRIL 11, 1953

3-Bromo-4-hydroxycoumarin (II) has been converted into various 3-thio-4-hydroxycoumarins. Of special interest is the preparation of 3-mercapto-4-hydroxycoumarin monohydrate (VII) which has been converted into a series of 3-(4-hydroxycoumarin)-α-thiocarboxylic acids (X), 2,3-dihydro-2,5-dioxo-5H-*p*-oxathiino[3,2-*c*][1]benzopyrans (XI), 3,3′-bis-(4-hydroxycoumarin) sulfide (VI) and disulfide (VIII). An improved synthesis of II is described.

3-Bromo-4-hydroxycoumarin (II) has previously been prepared in this Laboratory by the reaction of bromine with a suspension of 4-hydroxycoumarin (I) in chloroform.[2] This procedure was found to be tedious and the manipulation difficult for large scale synthesis. We have devised a modification of the original procedure which, in addition to being more convenient, gave a nearly quantitative yield of II.



3-Bromo-4-hydroxycoumarin reacted with thiourea under very mild conditions to yield 3-isothiourea-4-hydroxycoumarin hydrobromide (III). The free isothiourea derivative (IV) was readily prepared from the isothiouronium salt by dissolving III in hot water and allowing the product to crystallize from the water solution. Di-(3-isothiourea-4-hydroxycoumarin) sulfate (V) was prepared from either III or IV by reaction with concentrated sulfuric acid at room temperature. Compound V could be reconverted to IV by dissolving it in hot water. By heating V above its melting point, 3,3′-bis-(4-hydroxycoumarin) sulfide (VI) could be isolated. This compound also could be prepared by reaction of 3-isothiourea-4-hydroxycoumarin (IV) with 3-bromo-4-hydroxycoumarin (II), although only a low yield of VI was obtained.

By dissolving either III or IV in dilute sodium hydroxide solution, and then acidifying the solution with dilute hydrochloric acid, 3-mercapto-4-hydroxycoumarin monohydrate (VII) precipitated. The hydrate was very stable, remaining unchanged even after it was heated at 100° (3 mm.) over phos-

(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, together with that described in papers XIII and XIV of this series, is from the thesis submitted by Hugh R. Eisenhauer 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, June, 1953.

(2) C. F. Huebner and K. P. Link, THIS JOURNAL, **67**, 99 (1945).

TABLE I

3-(4-HYDROXYCOUMARIN)-α-THIOCARBOXYLIC ACIDS (X)

Acid[a]	Yield, %	M.p., °C.	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd	Hydrogen Found
R-α-Thioacetic	60	193–194	$C_{11}H_8O_5S$	52.4	52.3	3.2	3.4
R-α-Thiopropionic	48	192–194	$C_{12}H_{10}O_5S$	54.2	54.2	3.8	4.0
R-α-Thio-*n*-butyric	60	195–197	$C_{13}H_{12}O_5S$	55.7	55.6	4.3	4.5
R-α-Thio-*n*-valeric	65	171–173	$C_{14}H_{14}O_5S$	57.1	56.9	4.8	5.2
R-α-Thio-*n*-caproic	74	78–82	$C_{15}H_{16}O_5S$	58.4	58.0	5.2	5.3

[a] R = 3-(4-hydroxycoumarin).

TABLE II

2,3-DIHYDRO-2,5-DIOXO-5H-*p*-OXATHIINO[3,2-*c*][1]BENZOPYRANS (XI)

Benzopyran[a]	Yield, %	M.p., °C.	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
2,3-Dihydro-R	72	200–202	$C_{11}H_6O_4S$	56.4	56.5	2.6	3.2
2,3-Dihydro-3-methyl-R	85	165–167	$C_{12}H_8O_4S$	58.1	57.8	3.2	3.6
2,3-Dihydro-3-ethyl-R[b]	76	117–118	$C_{15}H_{16}O_5S$	58.5	58.4	5.2	5.5
2,3-Dihydro-3-*n*-propyl-R[b]	93	120–122	$C_{16}H_{18}O_5S$	59.7	59.5	5.6	5.8
2,3-Dihydro-3-*n*-butyl-R[b]	97	119–121	$C_{17}H_{20}O_5S$	60.8	61.3	6.0	6.3

[a] R = 2,5-Dioxo-5H-*p*-oxathiino[3,2-*c*][1]-benzopyran. [b] These compounds were isolated as the complex with one mole of ethanol

phorus pentoxide for 24 hours. This conversion was carried out in a nitrogen atmosphere since mercaptans are sensitive to air oxidation, especially in basic solution.[3] This was particularly evident by the fact that III, IV, V and VII all gave rise to 3,3′-bis-(4-hydroxycoumarin) disulfide (VIII) when their basic solutions were treated with acid in the presence of air. Compound VIII may also be prepared directly from 3-bromo-4-hydroxycoumarin (II) by reacting II with isothiourea in the presence of sodium ethylate.

It is of interest that acetylation of VII by the Chattaway procedure[4] yielded the monoacetate, 3-mercapto-4-acetoxycoumarin (IX). The fact that this was the isomer which was formed was shown by a positive Denigès' nitroprusside test,[5] indicating that a free sulfhydryl group was present.

3,3′-Bis-(4-hydroxycoumarin) sulfide (VI) was readily prepared from VII by reaction with 3-bromo-4-hydroxycoumarin (II) in the presence of sodium ethylate. 3-Mercapto-4-hydroxycoumarin monohydrate (VII) also reacted with a series of α-halo acids to yield the corresponding 3-(4-hydroxycoumarin)-α-thiocarboxylic acids (X, Table I). These were readily cyclized to give a series of 2,3-dihydro-2,5-dioxo-5H-*p*-oxathiino[3,2-*c*][1]benzopyrans (XI, Table II).

Experimental

3-Bromo-4-hydroxycoumarin (II).—Bromine (40 ml.) was added dropwise to an ice-cold solution of 4-hydroxycoumarin (100 g.) in absolute ethanol (1 liter). After allowing the reaction to proceed for 0.5 hour, this solution was added dropwise, with vigorous stirring, to ice and water (15 liters). The resulting slurry was kept at room temperature overnight. The crude 3-bromo-4-hydroxycoumarin was then filtered, washed with water and dried. The yield was 142 g. (96%). After recrystallization from ethyl acetate, the melting point was raised to 192–194°. A mixed melting point with an authentic sample of 3-bromo-4-hydroxycoumarin was undepressed.

3-Isothiourea-4-hydroxycoumarin Hydrobromide (III).—To a solution of 3-bromo-4-hydroxycoumarin (5 g.) in absolute ethanol (50 ml.) was added a solution of thiourea (1.9 g.) in absolute ethanol (50 ml.). After the reaction had proceeded for 24 hours at room temperature, the resulting white precipitate was filtered, washed with absolute ethanol and dried. The yield of 3-isothiourea-4-hydroxycoumarin hydrobromide was 5.0 g. (75%), m.p. 231–234°.

Anal. Calcd. for $C_{10}H_9BrN_2O_3S$: C, 37.9; H, 2.8; Br, 25.2. Found: C, 37.8; H, 3.0; Br, 24.3.

3-Isothiourea-4-hydroxycoumarin (IV).—Either III or V (4.8 g.) was dissolved in boiling water (500 ml.). On cooling, 3-isothiourea-4-hydroxycoumarin crystallized from the water solution. The yield of IV from III was 3.5 g. (98%) and from V, 2.4 g. (62%). The melting point of the product was raised to 216–217° by recrystallization from water.

Anal. Calcd. for $C_{10}H_8N_2O_3S$: C, 50.8; H, 3.4. Found: C, 50.5; H, 4.0.

Di-(3-isothiourea-4-hydroxycoumarin) Sulfate (V).—Either III or IV (2.4 g.) was added in small portions at room temperature to concentrated sulfuric acid (24 ml.). After complete addition, the clear red solution was poured, with stirring, into ice-water (200 ml.). The product, which soon crystallized from the dilute acid, was filtered, washed with water and dried. The yield of di-(3-isothiourea-4-hydroxycoumarin) sulfate from III was 2.0 g. (93%) and from IV, 2.6 g. (90%), m.p. 182° dec.

Anal. Calcd. for $C_{20}H_{18}N_4O_{10}S_3$: C, 42.1; H, 3.2. Found: C, 41.8; H, 3.4.

3,3′-Bis-(4-hydroxycoumarin) Sulfide (VI). (a) **From Di-(3-isothiourea-4-hydroxycoumarin) Sulfate (V).**—V (0.95 g.) was heated at 185–195° for 0.5 hour. The residue was then triturated with a small amount of 5% sodium hydroxide solution and filtered. The filtrate, on acidification with dilute hydrochloric acid solution, yielded 3,3′-bis-(4-hydroxycoumarin) sulfide (0.25 g., 39%). On recrystallization from ethyl acetate, the melting point and mixed melting point with an authentic sample of VI[6] was 317–318° (metal block).

(b) **From 3-Isothiourea-4-hydroxycoumarin (IV).**—A solution of 3-isothiourea-4-hydroxycoumarin (0.75 g.) and 3-bromo-4-hydroxycoumarin (0.50 g.) in absolute ethanol (100 ml.) was refluxed for three hours. The reaction mixture was then cooled and evaporated to dryness *in vacuo*. The residue was heated with water (100 ml.) and filtered. The undissolved solid (0.10 g., 14%) was recrystallized from ethyl acetate to yield pure VI, m.p. 314–318°. A mixed melting point with authentic 3,3′-bis-(4 hydroxycoumarin) sulfide was not depressed.

(3) R. C. Fuson, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 573.

(4) F. D. Chattaway, *J. Chem. Soc.*, 2495 (1931).

(5) S. P. Milliken, "A Method for the Identification of Pure Organic Compounds," Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1922, p. 17.

(6) 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, *J. Biol. Chem.*, **153**, 5 (1944).

(c) From **3-Mercapto-4-hydroxycoumarin Monohydrate** (VII).—To a refluxing solution of 3-mercapto-4-hydroxycoumarin monohydrate (1.0 g.) and 3-bromo-4-hydroxycoumarin (1.7 g.) in absolute ethanol (50 ml.), through which dry nitrogen gas was passing, was added a solution of sodium (0.6 g.) in absolute ethanol (50 ml.). After refluxing the reaction mixture for three hours, it was evaporated to a volume of about 25 ml. The mixture was then added to ice and hydrochloric acid. The product was filtered, washed with water and dried. Recrystallization from ethyl acetate yielded 3,3′-bis-(4-hydroxycoumarin) sulfide (1.2 g., 72%). The melting point and mixed melting point with authentic 3,3′-bis-(4-hydroxycoumarin) sulfide was 315–320°.

3-Mercapto-4-hydroxycoumarin Monohydrate (VII).—A six-liter 3-necked round-bottom flask, fitted with a mechanical stirrer, dropping funnel and inlet tube for nitrogen gas, was charged with water (800 ml.). The system was swept out with nitrogen gas for 0.5 hour under stirring. Subsequently either finely powdered III (31.7 g.) or IV (23.6 g.) was slowly added. Upon complete addition, a 5% sodium hydroxide solution (250 ml.) was added dropwise to yield a clear yellow solution. The stirring and nitrogen flushing was continued for an additional 0.5 hour. A 5% hydrochloric acid solution (300 ml.) was then added dropwise to the reaction mixture. The resulting light yellow precipitate was filtered, washed with water and finally dried in a vacuum desiccator. The yield of 3-mercapto-4-hydroxycoumarin monohydrate from III was 17.2 g. (81%) and from IV was 16.0 g. (75%). Recrystallization of the product, which gave a positive Denigès' nitroprusside test,[5] from ethanol or ethanol–ether raised the melting point to 210° dec.

Anal. Calcd. for $C_9H_6O_3S\cdot H_2O$: C, 50.9; H, 3.8. Found: C, 50.3; H, 3.9.

3,3′-Bis-(4-hydroxycoumarin) Disulfide (VIII). (a) From III, IV or V.—Compound III (29.3 g.), IV (3.5 g.) or V (0.3 g.) was dissolved in a slight excess of 1% sodium hydroxide solution. The reaction was allowed to proceed at room temperature for one hour. The solution was then acidified with dilute hydrochloric acid to yield a light yellow, gelatinous precipitate. The product which was filtered, washed with water and dried, melted at 285–290° dec. The yield of 3,3′-bis-(4-hydroxycoumarin) disulfide from III was 14.9 g. (83%), from IV, 1.8 g. (63%) and from V, 0.15 g. (74%). A satisfactory solvent for the recrystallization of the disulfide VIII could not be found. Accordingly, the product made from analytically pure 3-isothiourea-4-hydroxycoumarin was used for the analysis indicated below.

Anal. Calcd. for $C_{18}H_{10}O_6S_2$: C, 55.9; H, 2.6. Found: C, 55.3; H, 3.1.

(b) From **3-Mercapto-4-hydroxycoumarin Monohydrate**.—Air was bubbled through a solution of VII (0.25 g.) in a 1% sodium hydroxide solution (25 ml.) for one hour. The solution was then acidified with dilute hydrochloric acid to yield 3,3′-bis-(4-hydroxycoumarin) disulfide (0.20 g., 87%) having a melting point of 290–295°. A mixed melting point with authentic VIII was not depressed. Compound VIII gave a negative Denigès' nitroprusside test.[5]

(c) From **3-Bromo-4-hydroxycoumarin**.—Compound II (3.0 g.) and thiourea (1.1 g.) were dissolved in absolute ethanol (50 ml.). To this solution was added a solution of sodium (0.35 g.) in absolute ethanol (50 ml.). The reaction mixture was refluxed for two hours. The reaction mixture was then diluted with water (400 ml.) and acidified with dilute hydrochloric acid to yield 3,3′-bis-(4-hydroxycoumarin) disulfide (1.45 g., 60%). The melting point and mixed melting point with authentic VIII was 290–300°.

3,3′-Bis-(4-hydroxycoumarin) Disulfide, Dimethyl Ether.—Excess diazomethane in diethyl ether was added to a suspension of 3,3′-bis-(4-hydroxycoumarin) disulfide (1.0 g.) in ether (25 ml.). The mixture was kept at room temperature for 0.5 hours after the evolution of nitrogen had ceased. The precipitate was filtered and washed with ether. The crude product was then triturated with 1% sodium hydroxide solution, filtered, washed with water and dried. The product was recrystallized from ethyl acetate. The yield of 3,3′-bis-(4-hydroxycoumarin) disulfide dimethyl ether was 0.4 g. (37%), m.p. 194–197°.

Anal. Calcd. for $C_{20}H_{14}O_6S_2$: C, 58.0; H, 3.4. Found: C, 57.9; H, 3.7.

3-Mercapto-4-acetoxycoumarin (IX).—A mixture of 3-mercapto-4-hydroxycoumarin monohydrate (1.0 g.) and water (10 ml.), through which nitrogen gas was bubbled, was stirred vigorously. A 10% sodium hydroxide solution (10 ml.) was then added dropwise. The clear yellow solution was cooled to 0° in an ice–salt-bath, and acetic anhydride (5 ml.) was added in one portion. After ten minutes, the solution was acidified with dilute hydrochloric acid and the resulting light yellow precipitate was filtered, washed with water and dried. The yield of 3-mercapto-4-acetoxycoumarin was 0.8 g. (71%). The Denigès' nitroprusside test[5] was positive indicating that a free sulfhydryl group was present. The melting point of IX was raised to 180–182° by recrystallization from acetone–Skellysolve B (1:10).

Anal. Calcd. for $C_{11}H_8O_4S$: C, 56.0; H, 3.4. Found: C, 56.1; H, 3.6.

3-(4-Hydroxycoumarin)-α-thiocarboxylic Acids (X).—The synthesis of X was similar in all cases and may be illustrated by the preparation of 3-(4-hydroxycoumarin)-α-thio-*n*-butyric acid (X, R = C_2H_5). To a refluxing solution of 3-mercapto-4-hydroxycoumarin hydrate (1.0 g.) and α-bromo-*n*-butyric acid (2.0 ml.) in absolute ethanol (125 ml.) which was being continuously swept with dry nitrogen gas, was added a solution of sodium (0.6 g.) in absolute ethanol (25 ml.). The reaction mixture was refluxed for three hours. The resulting solution was then evaporated to about 10 ml. and diluted with water (50 ml.). The clear yellow solution was acidified with dilute hydrochloric acid to yield a voluminous precipitate which was filtered, washed with water and dried. The product was recrystallized from water. The yield and analysis of the various 3-(4-hydroxycoumarin)-α-thiocarboxylic acids are indicated in Table I.

2,3-Dihydro-2,5-dioxo-5H-*p*-oxathiino[3,2-*c*][1]benzopyrans (XI).—The synthesis of XI was similar in all cases and may be illustrated by the preparation of XI, R = C_2H_5. To a suspension of 3-(4-hydroxycoumarin)-α-thio-*n*-butyric acid (0.35 g.) in dry benzene (50 ml.) was added thionyl chloride (2 ml.). The reaction mixture was refluxed for one hour. After complete reaction, the clear yellow solution was evaporated to a volume of about 5 ml. Absolute ethanol (50 ml.) was added and the resulting solution was again evaporated to a volume of about 5 ml. The solution was then added dropwise, with vigorous stirring, to ice-water (150 ml.) to yield a voluminous, light yellow precipitate. (It was more expedient to isolate compounds XI, R = H and CH_3, by adding Skellysolve B to the concentrated benzene reaction mixture.) The product was recrystallized from ethanol–water, or, in the case of XI, R = H and CH_3, from benzene–Skellysolve B. It should be noted that for compounds XI, R = C_2H_5, *n*-C_3H_7 and *n*-C_4H_9, the product from recrystallization was isolated as the complex with one mole of ethanol. This complex could not be destroyed by heating at 60° (3 mm.) over phosphorus pentoxide for 24 hours. The yield and analysis of the various compounds of this series, all of which were insoluble in dilute sodium hydroxide solution, is indicated in Table II.

Acknowledgment.—We are indebted to our colleague, Mr. Collin H. Schroeder for all the carbon and hydrogen analyses. We also wish to express our gratitude to Professor A. L. Wilds of the Chemistry Department of this University for his helpful advice and suggestions; to Dr. Austin M. Patterson, Xenia, Ohio, and Dr. L. T. Capell of the staff of "Chemical Abstracts" for their advice on the nomenclature used.

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