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

Studies on 4-Hydroxycoumarins. II. The Condensation of Aldehydes with 4-Hydroxycoumarins[1]

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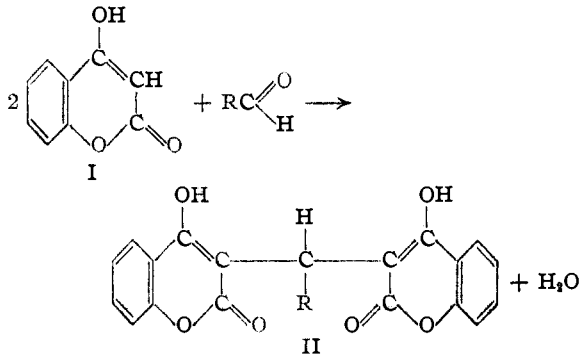
In a study of the relationship between chemical structure and anticoagulant activity,[2] we have prepared compounds containing substituent groups on the methylene carbon atom of 3,3′-methylene bis-(4-hydroxycoumarin) by condensing 4-hydroxycoumarin with aldehydes other than formaldehyde. The general reaction is



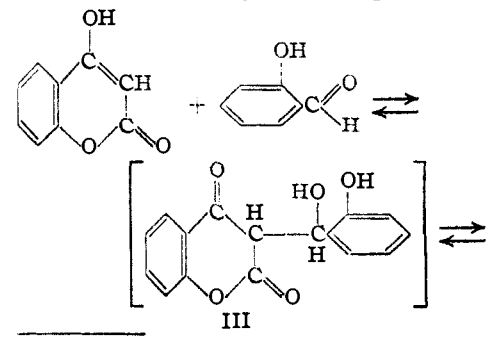
Wolff[3] observed that two moles of tetronic acid (γ-hydroxyacetoacetic acid lactone) would condense with one mole of an aldehyde or a ketone to produce substituted *bis*-tetronic acids. Anschütz[4] reported the condensation of "benzotetronsäure" (4-hydroxycoumarin, the enol of *o*-hydroxybenzoylacetic acid lactone) with formaldehyde and with acetaldehyde in aqueous solution, but was unable to effect condensation with propionaldehyde, butyraldehyde or acetone under similar conditions. The compounds described in this report were obtained by carrying out the reactions in an organic solvent.

These *bis*-4-hydroxycoumarins are colorless, crystalline solids. Due to the presence of the enolic hydroxyl groups they dissolve in alkali to form dibasic salts, are readily methylated by diazomethane, and form diesters.[5] Heating with alkali opens the lactone rings and subsequent ketonic cleavage yields 1,5-diketones.[6,7] Heating with aniline at 180° produces the anil of 4-hydroxycoumarin.[6] They can be dehydrated to form substituted 1,4-pyrans,[8] the loss of water occurring between the enolic hydroxyls at positions 4 and 4′.

The condensation of 4-hydroxycoumarin with the two *o*-hydroxybenzaldehydes studied gave rise to products which may indicate the course of the reaction. In the discussion that follows, the results with salicylaldehyde are given special consideration.

When an equimolar mixture of 4-hydroxycoumarin and salicylaldehyde was refluxed for ten minutes in ethanolic solution, bright yellow crystals of 3-(*o*-hydroxybenzal)-2,4-diketochroman (IV) separated on cooling. Its yellow color is probably due to a quinoid chromophore. Analogous products have been prepared by Sonn[9] by condensing anisaldehyde with 4,7-dihydroxy- and 4,5,7-trihydroxycoumarins. The condensation is of the "aldol" type as defined by Hauser and Breslow[10] and probably involves an intermediate hydroxy compound (III).

When the molar ratio of 4-hydroxycoumarin to salicylaldehyde was increased to two to one, and the time of heating increased to an hour or longer, the major product contained two 4-hydroxycoumarin residues. This product, 3-[6-oxo(1)benzopyrano(4,3-*b*) - (1)benzopyran-7-yl] - 4 - hydroxycoumarin (VI), was apparently formed by the loss of water from 3,3′-(*o*-hydroxybenzylidene)-bis-(4-hydroxycoumarin) (V). Furthermore, when a mixture of IV and 4-hydroxycoumarin was refluxed in ethanolic solution, this same product VI was obtained. These results are analogous to those of Breslow and Hauser[11] on the condensation of benzaldehyde with ethyl malonate, from which they concluded that the formation of ethyl benzaldimalonate probably involves the intermediate formation of ethyl benzalmalonate. Hence it may be postulated that the condensation of salicylaldehyde with 4-hydroxycoumarin proceeds as follows



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(2) A manuscript dealing with the biological activity of compounds related to 3,3′-methylenebis-(4-hydroxycoumarin) is in preparation and will appear elsewhere.

(3) Wolff, *Ann.*, **315**, 145 (1901).

(4) Anschütz, *ibid.*, **367**, 217 (1909).

(5) Stahmann, Graf, Huebner, Roseman and Link, Paper IV of this series to appear later.

(6) Stahmann, Huebner and Link, *J. Biol. Chem.*, **138**, 513 (1941).

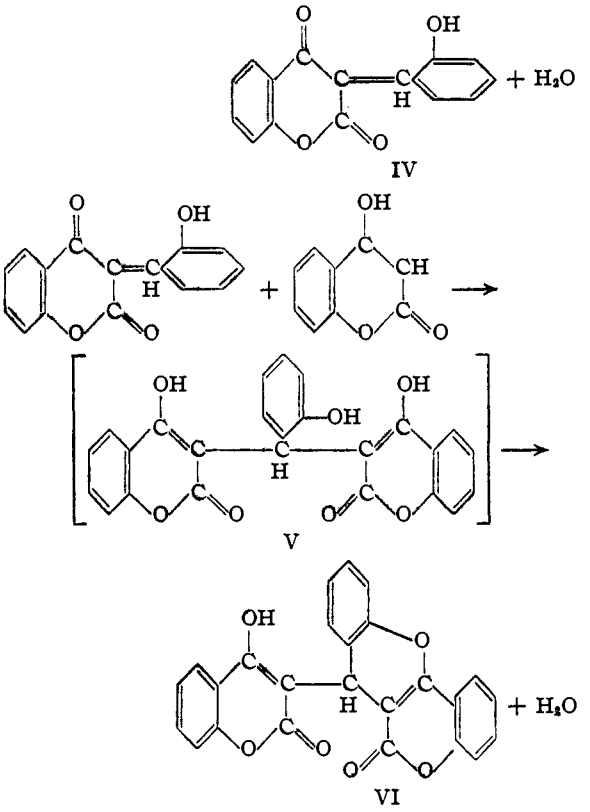
(7) Huebner and Link, *ibid.*, **138**, 529 (1941).

(8) Huebner, Sullivan, Stahmann and Link, THIS JOURNAL, **65**, 2292 (1943).

(9) Sonn, *Ber.*, **50**, 1292 (1917).

(10) Hauser and Breslow, THIS JOURNAL, **62**, 2389 (1940).

(11) Breslow and Hauser, *ibid.*, **62**, 2385 (1940).



The structure proposed for the final product is based on the following considerations. Elementary analysis and electrometric titration indicated the loss of water between two hydroxyl groups of the expected product (V). This might have involved either the two enolic hydroxyl groups of the 4-hydroxycoumarin residues or one of these and the phenolic hydroxyl on the *o*-hydroxybenzylidene residue. The anticoagulant activity of the product[2] and the fact that it is cleaved by aniline at 180° to give the anil of 4-hydroxycoumarin eliminate the former possibility, since these properties are not shown by 3,3′-alkylidene-4,4′-epoxydicoumarins.

The condensation of IV with 4-hydroxycoumarin is of the Michael type.[10] It is an unusual example because no added catalyst is required, while most Michael reactions require either a basic or an acidic[11] catalyst. To make certain that this type of condensation actually occurs, IV was treated with 6-methyl-4-hydroxycoumarin. The product obtained gave correct carbon and hydrogen analyses for VI with a methyl group substituted into the molecule. Such a product could only have been formed by the addition of one molecule of 6-methyl-4-hydroxycoumarin to IV.

Attempts to purify IV by repeated recrystallization to a constant melting point always gave the high melting bis product (VI). Therefore, microscopic appearance and sharpness of melting point were used as criteria of homogeneity and a sample melting sharply at 173–174° was taken for analysis. This analysis was in agreement with Formula IV. To show that conversion of IV to VI occurred during the recrystallization (rather than separation of IV from VI), an ethanolic solution of IV was refluxed for several hours. Considerable loss of color was noted and the colorless bis product was recovered in 73% yield. Apparently the initial aldol condensation is reversible and the 4-hydroxycoumarin so formed then undergoes the Michael addition with some of the unchanged IV.

Experimental

Condensation of Aldehydes with 4-Hydroxycoumarin.—The aldehydes (E. K. Co., redistilled) were added to a 20% solution of 4-hydroxycoumarin[12] in hot ethanol and the mixture refluxed for an hour. An excess of aldehyde (up to 50% above the theoretical amount) was used in the experiments reported here. The products usually separated on cooling and were removed by filtration. Additional amounts of the condensation products were obtained by concentrating the mother liquors or by adding water. The crops were combined and recrystallized until the melting points remained constant.

Variations from this general procedure are noted in Table I. Thus, in some cases it was found advisable to add more aldehyde to the mother liquors from the first crop and heat again. In the case of *n*-hexaldehyde the use of aluminum chloride as catalyst was found desirable.

Methyl ethers were prepared by distilling an excess of diazomethane into solutions or suspensions of the condensation products in dry ether. After standing in the hood twenty-four hours, the ether and remaining diazomethane were removed by distillation and the products recrystallized from ethanol.

3-[6-Oxo(1)benzopyrano(4,3-*b*)-(1)benzopyran-7-yl]-4-hydroxycoumarin (VI).—Five grams (0.031 mole) of 4-hydroxycoumarin was dissolved in 25 ml. of hot ethanol, 2.3 g. (0.019 mole) of salicylaldehyde added and the mixture refluxed for an hour. From the cooled mixture, 5.48 g. of solid was filtered off, which consisted of a mixture of yellow needles and colorless prisms. After three recrystallizations from ethanol, colorless prisms (2.8 g., 44%) were obtained, m. p. 242° dec. Further recrystallization raised the melting point to 245° dec. The electrometric titration curve showed one deflection, at *p*H 5.7. Treatment with aniline at 180° gave the anil of 4-hydroxycoumarin, m. p. 262–263°.

Anal. Calcd. for $C_{25}H_{14}O_6$: C, 73.17; H, 3.41. Found: C, 73.44; H, 3.51.

3-[6-Oxo(1)benzopyrano(4,3-*b*)-(1)10-hydroxybenzopyran-7-yl]-4-hydroxycoumarin.—4-Hydroxycoumarin (5 g., 0.03 mole) was dissolved in 25 ml. of hot ethanol, 2 g. (0.0145 mole) of 2,4-dihydroxybenzaldehyde added and the solution refluxed one hour. From the cooled reaction mixture, 3.85 g. of solid was filtered off. Cooling the mother liquor in the icebox gave a second crop (total yield 4.1 g., 60%). After recrystallization from glacial acetic acid the needles melted at 251°, dec. Electrometric titration indicated two acidic groups and degradation by heating with aniline gave the anil of 4-hydroxycoumarin.

Anal. Calcd. for $C_{25}H_{14}O_7$: C, 70.42; H, 3.60. Found: C, 70.16; H, 3.28.

The **acetate** was prepared by treating 1.4 g. of the product, dissolved in 5 ml. of warm pyridine, with 5 ml. of acetic anhydride. Addition of water to the cooled solutionca used precipitation of the acetate. After recrystallization from glacial acetic acid, it melted at 236°, with decomposition.

Anal. Calcd. for $C_{25}H_{12}O_7(COCH_3)$: C, 68.23; H, 3.53; $COCH_3$, 16.86. Found: C, 68.11; H, 3.55; $COCH_3$, 16.70.

(12) Prepared by the method of Stahmann, *et al.*, THIS JOURNAL, 65, 2285 (1943).

TABLE I

ALDEHYDE CONDENSATION PRODUCTS OF 4-HYDROXYCOUMARIN

Compound	Yield, %	M. p., °C. (uncor.)	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Methoxyl Calcd.	Methoxyl Found
3,3′-Ethylidenebis-(4-hydroxycoumarin)[a]	67	176–178	$C_{20}H_{12}O_6$						
3,3′-Propylidene-(4-hydroxycoumarin). a. Dimethyl ether	69	144–145 129	$C_{21}H_{16}O_6$ $C_{21}H_{14}O_4(OCH_3)_2$	69.23	69.61	4.40	4.70	15.81	15.49
3,3′-*n*-Butylidenebis-(4-hydroxycoumarin).[b] a. Dimethyl ether	86	123–124 118–120	$C_{22}H_{18}O_6$ $C_{22}H_{16}O_4(OCH_3)_2$	69.84	69.69	4.76	5.04	15.27	15.04
3,3′-Isobutylidenebis-(4-hydroxycoumarin).[c] a. Dimethyl ether	78	199–200 214–215	$C_{22}H_{18}O_6$ $C_{22}H_{16}O_4(OCH_3)_2$	69.84	69.91	4.76	4.76	15.27	14.80
3,3′-*n*-Pentylidenebis-(4-hydroxycoumarin).[d] a. Dimethyl ether	75	113 129–130	$C_{23}H_{20}O_6$ $C_{23}H_{18}O_4(OCH_3)_2$	70.41	69.87	5.10	5.10	14.76	14.58
3,3′-Isopentylidenebis-(4-hydroxycoumarin).[e] a. Dimethyl ether	50	142–143 148	$C_{23}H_{20}O_6$ $C_{23}H_{18}O_4(OCH_3)_2$	70.41 71.43	70.41 71.50	5.10 5.71	5.04 5.71	14.76	14.40
3,3′-*n*-Hexylidenebis-(4-hydroxycoumarin).[f] a. Dimethyl ether	18	104–105 113–115	$C_{24}H_{22}O_6$ $C_{24}H_{20}O_4(OCH_3)_2$	70.94	71.03	5.42	5.32	14.29	14.12
3,3′-Benzylidenebis-(4-hydroxycoumarin). a. Dimethyl ether	91	228–229 181–183	$C_{25}H_{16}O_6$ $C_{25}H_{14}O_4(OCH_3)_2$	72.82 73.64	72.69 73.67	3.88 4.55	4.03 4.69	14.09	13.81
3,3′-Phenylethylidenebis-(4-hydroxycoumarin)[g]	40	175–177	$C_{26}H_{18}O_6$	73.24	73.18	4.22	4.28		
3,3′-Phenylpropylidenebis-(4-hydroxycoumarin).[h] a. Dimethyl ether	85	197–198 170–173	$C_{27}H_{20}O_6$ $C_{27}H_{18}O_4(OCH_3)_2$	73.64	73.49	4.55	4.55	13.25	12.98
3,3′-(*p*-Methoxybenzylidine)-bis-(4-hydroxycoumarin).[i] a. Dimethyl ether	80	242 dec. 170–171	$C_{25}H_{15}O_6(OCH_3)$ $C_{25}H_{13}O_4(OCH_3)_3$	70.59 70.41	70.62 70.54	4.07 5.10	4.27 5.13	7.05	6.94
3,3′-(*p*-Hydroxy-*m*-methoxybenzylidene)-bis-(4-hydroxycoumarin)[j]	93	213–215	$C_{25}H_{17}O_7(OCH_3)$	68.12	67.91	3.93	3.97	6.77	6.90
3,3′-(*m*,*p*-Methylenedioxybenzylidene)-bis-(4-hydroxycoumarin)[k]	67	256 dec.	$C_{26}H_{16}O_8$	68.42	68.36	3.51	3.56		
3,3′-(*p*-Dimethylaminobenzylidene)-bis-(4-hydroxycoumarin)[l]	76	210 dec.	$C_{27}H_{21}O_6N$	71.21	71.52	4.62	4.73		
3,3′-Carboxymethylenebis-(4-hydroxycoumarin).[m] a. Dimethyl ether methyl ester	76	244–245 160–161	$C_{20}H_{12}O_8$ $C_{20}H_9O_5(OCH_3)_3$	63.16	63.14	3.17	3.22	22.05	22.00
3,3′,3″,3‴-Adipylidene-tetrakis-(4-hydroxycoumarin).[n] a. Tetramethyl ether[o]	38	219–220 230–232	$C_{42}H_{30}O_{12}$ $C_{42}H_{26}O_8(OCH_3)_4$	69.42	69.35	4.14	4.31	15.66	15.46

[a] Anschütz[4] reported a product melting at 165° from 4-hydroxycoumarin and acetaldehyde. Our product showed a higher melting point after recrystallization from ethanol and dioxane. [b] Reaction mixture refluxed for two hours, cooled, filtered, additional aldehyde added and refluxed again for three hours. [c] Refluxed initially for four hours, cooled, filtered, additional aldehyde added and refluxed again for two hours. This second refluxing was repeated. Recrystallized from ethanol–dioxane (6:1). [d] Refluxed initially for two and one-half hours, cooled, filtered, additional aldehyde added and then refluxed for two hours. [e] Reaction mixture refluxed for two hours. [f] Aluminum chloride (molar ratio of 4-hydroxycoumarin to $AlCl_3$, 4:1) was added to a solution of the reactants in dioxane in a large test-tube and heated at 135° for thirty minutes. The remaining oil was taken up in hot alcohol, filtered to remove alumina and crystallization induced by cooling and stirring. [g] The reaction mixture was refluxed for one hour, cooled, filtered, and the filtrate again refluxed for one hour. The product was recrystallized from ethanol–acetone mixtures. [h] Recrystallized from benzene. [i] Recrystallized from ethyl ether. [j] Reaction mixture refluxed for half an hour. Product recrystallized from cyclohexanone. [k] Recrystallized from cyclohexanone. [l] Soluble in alkali and acid but could not be recrystallized from any of several neutral solvents tried. The washed crude product was analyzed. *Anal.* Calcd. for $C_{27}H_{21}O_6N$: N, 3.07. Found: N, 3.00. [m] An aqueous solution of glyoxalic acid was prepared by the method of Benedict.[13] 4-Hydroxycoumarin was refluxed with this solution. [n] Adipic aldehyde was prepared by oxidation of *trans*-cyclohexanediol-1,2 with lead tetraacetate. This was condensed with 4-hydroxycoumarin in ethanol containing a trace of oxalic acid. The crude sirup was extracted with hot water to remove unreacted 4-hydroxycoumarin, then taken up in hot ethanol. On cooling crystals separated which were recrystallized from cyclohexanone. [o] Recrystallized from glacial acetic acid.

The **methyl ether** was prepared with diazomethane as described above, and recrystallized from dioxane–water mixtures, m. p. 301–304° with decomposition.

Anal. Calcd. for $C_{25}H_{13}O_6(OCH_3)$: OCH_3, 7.05. Found: OCH_3, 7.36.

3-(*o*-Hydroxybenzal)-2,4-diketochroman (IV).—4-Hydroxycoumarin (9.72 g., 0.06 mole) was dissolved in 100 ml. of boiling ethanol, 7.32 g. (0.06 mole) of salicylaldehyde added, and the solution refluxed for ten minutes. After standing for one hour at 25°, a portion of yellow needles was filtered off and washed with ethanol. After drying overnight in a vacuum desiccator, this material weighed 1.80 g. and consisted of practically pure IV, m. p. 173–174°. Cooling the mother liquors overnight in the ice-box gave 2.42 g. of a mixture of the two condensation products (IV and VI) which was removed by filtration. The mother liquor gave a test for unreacted 4-hydroxycoumarin

(13) Hawk and Bergeim, "Practical Physiological Chemistry," Blakiston Co., Philadelphia, 1937, p. 926.

(precipitate with formaldehyde). More salicylaldehyde (7.32 g.) was added and the solution refluxed for ten minutes. After an hour at 25° another portion of IV was obtained which when dry weighed 1.40 g. and melted at 175°. The total yield was 3.20 g. (20%).[14]

Anal. Calcd. for $C_{16}H_{10}O_4$: C, 72.18; H, 3.76. Found: C, 72.22; H, 3.87.

A **semicarbazone** was prepared which after recrystallization from ethanol melted at 220–221°.

Anal. Calcd. for $C_{17}H_{13}O_4N_3$: N, 13.0. Found: N, 12.9.

3-(*o,p*-Dihydroxybenzal)-2,4-diketochroman.—4-Hydroxycoumarin (9.72 g., 0.06 mole) was dissolved in 100 ml. of hot ethanol, 8.28 g. (0.06 mole) of 2,4-dihydroxybenzaldehyde added, and the solution refluxed for ten minutes. Successive crops of solid were obtained by cooling, filtering and concentrating the mother liquors. Finally water was added and unreacted aldehyde (2.43 g.) crystallized out. After extraction of the combined fractions with boiling ethanol the colorless bis condensation product (4.79 g.) remained. From the ethanol soluble fraction was obtained about 1 g. of unreacted 4-hydroxycoumarin and 3.87 g. (23%) of 3-(*o,p*-dihydroxybenzal)-2,4-diketochroman, yellow needles decomposing at 224°.

Anal. Calcd. for $C_{16}H_{10}O_5$: C, 68.08; H, 3.54. Found: C, 68.24; H, 3.58.

Condensation of 4-Hydroxycoumarin with 3-(*o*-Hydroxybenzal)-2,4-diketochroman (IV).—IV (0.532 g., 0.002 mole) and 4-hydroxycoumarin (0.324 g., 0.002 mole) were dissolved in 25 ml. of hot ethanol and the solution refluxed for five hours. By this time the yellow color had almost disappeared. Cooling in the icebox caused precipitation of colorless prisms which were removed by filtration, washed with ethanol and dried. The product melted at 241–243°, dec., and was identical with the *bis* condensation product (VI). The yield was 0.626 g. (76.3%).

Condensation of 6-Methyl-4-hydroxycoumarin with 3-(*o*-Hydroxybenzal)-2,4-diketochroman (IV).—IV (0.532 g., 0.002 mole) and 6-methyl-4-hydroxycoumarin (0.352 g., 0.002 mole; prepared by the method of Anschütz,[15] m. p. 240–250°) were dissolved in 25 ml. of hot ethanol and the solution refluxed for five hours. After cooling in the icebox, 0.4352 g. of solid, m. p. 267–275°, dec., was obtained by filtration. Adding 20 ml. of water to the mother liquor gave a further 0.089 g., m. p. 267–269° with decomposition. The total yield was 0.5242 g. (61.7%). Two recrystallizations from dioxane–water mixtures gave colorless prisms which melted at 277–278°, dec.

Anal. Calcd. for $C_{26}H_{16}O_6$: C, 73.58; H, 3.77. Found: C, 73.58; H, 4.10.

Conversion of 3-(*o*-Hydroxybenzal)-2,4-diketochroman (IV) to 3-[6-Oxo(1)benzopyrano(4,3-*b*)-(1)benzopyran-7-yl]-4-hydroxycoumarin (VI) by Heating in Ethanolic Solution.—IV (0.8 g.) was dissolved in 25 ml. of hot ethanol and the solution heated thirteen and one-half hours on the steam-bath under reflux. After cooling in the icebox, the colorless prisms were filtered off, washed with ethanol and dried. The crude product melted at 240–241° dec. and after recrystallization from ethanol melted at 245° dec. The mixed melting point with an authentic sample of VI showed no depression. The yield, 0.4503 g., was 73.2% of the theoretically possible amount.

Acknowledgment.—We are indebted to our colleagues, Mr. Lloyd Graf and Mr. Lewis Fauble, for some of the analyses herein reported and to Dr. Austin M. Patterson for his generous advice on nomenclature.

Summary

1. 4-Hydroxycoumarin has been condensed with several aliphatic and aromatic aldehydes and the products described.

2. The 3,3′-arylidenebis-(4-hydroxycoumarin)s from two *o*-hydroxybenzaldehydes undergo spontaneous dehydration and ring closure to form substituted benzopyrans. Colored "intermediate" compounds representing the condensation of one molecule of *o*-hydroxybenzaldehyde with one molecule of 4-hydroxycoumarin were obtained in these two cases.

3. 4-Hydroxycoumarin adds to 3-(*o*-hydroxybenzal)-2,4-diketochroman on heating in ethanolic solution. This addition is analogous to the Michael reaction and proceeds without the addition of a catalyst.

4. 3-(*o*-Hydroxybenzal)-2,4-diketochroman is converted to 3-[6-oxo(1)benzopyrano(4,3-*b*)-(1)-benzopyran-7-yl]-4-hydroxycoumarin when heated in ethanolic solution.

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(14) IV can be recovered from the mixtures by extracting with ethyl ether and recrystallizing from ether until the material melts at 174–175°. However, this process is unsatisfactory and in the method given here it is most economical to add the mixtures to the mother liquor and convert all to the bis product by refluxing for several hours.

(15) Anschütz, *Ann.*, **367**, 251 (1909).