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

Studies on 4-Hydroxycoumarins. I. The Synthesis of 4-Hydroxycoumarins[1]

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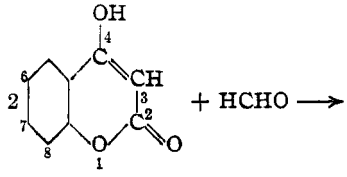
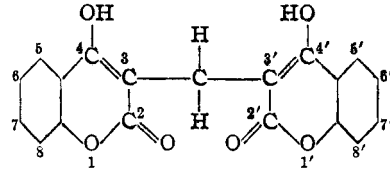
3,3'-Methylenebis-(4-hydroxycoumarin) (II) the causative agent of the hemorrhagic sweet clover disease of cattle[2,3,4,5,6] is readily synthesized by condensing formaldehyde with 2 molecular equivalents of 4-hydroxycoumarin (I).



I



II

This paper describes an improved synthesis of 4-hydroxycoumarin from methyl acetylsalicylate, and the application of this method of preparation to the synthesis of 3-substituted-4-hydroxycoumarins.

Anschütz[7] first synthesized 4-hydroxycoumarin by treating acetylsalicylyl chloride with the sodium derivative of malonic ester to form 3-carboethoxy-4-hydroxycoumarin. On treatment with alkali this compound was decarboxylated to form 4-hydroxycoumarin.

Sonn[8] and Bauer and Schoder[9] applied the Hoesch synthesis[10] to the preparation of 4-hydroxycoumarin having hydroxyl substituents on the benzene ring. By condensing cyanoacetic ester with resorcinol or phloroglucinol in the presence of hydrochloric acid and zinc chloride, followed by hydrolysis of the intermediate ketimide, the corresponding substituted 4-hydroxycoumarin was formed. Attempts were made to condense cyanoacetic ester with cresols, hydroquinone, and phenol but the ketimides could not be obtained.

Pauly and Lockemann[11] synthesized 4-hydroxycoumarin from methyl acetylsalicylate by adding metallic sodium to the molten ester. They reported a yield of 55%. However, by following their conditions we were not able, in spite of many trials, to obtain pure 4-hydroxycoumarin in yields above 13%. Appreciable quantities of other acidic products are formed in this reaction and it is our belief that the yield indicated by them was based on very crude preparations.

We have found that this reaction could be brought under better control in an inert solvent and subsequently we studied the effect of solvent, temperature, time of reaction, type and amount of condensing agent, order of adding reactants, rate of mixing, type of ester, and other conditions on the yields of 4-hydroxycoumarin. When an inert solvent was used, a broader temperature range was permissible with the optimum, 240–250°, considerably above the maximum of 175° specified by Pauly and Lockemann. The reaction product can then be recovered as a granular powder.

Other condensing agents studied for this intramolecular Claisen condensation were sodium methylate, sodium ethylate, sodium isopropylate, and sodium amylate. With them the same temperature range 240–250° was required for the reaction. The yields obtained with the alcoholates were generally lower than with metallic sodium. Potassium reacted more vigorously than sodium and the reaction could be carried out at a lower temperature. Sodamide effected the condensation but appeared to offer no advantage over metallic sodium. The amount of condensing agent or the ratio of reactants was not critical, for the sodium could be considerably in excess or much lower than the theoretical without greatly affecting the yields.

4-Hydroxycoumarins having a substituent group on carbon 3 (III) were obtained by acylating methyl salicylate (IV) with the acid anhydrides or chlorides of organic acids having two hydrogen atoms on the alpha carbon (V) and then treating the resulting ester (VI) with sodium. Thus 3-methyl-4-hydroxycoumarin was obtained from methyl propionylsalicylate by a procedure similar to that for 4-hydroxycoumarin. Ethyl acetylsalicylate also formed 4-hydroxycoumarin when treated with sodium.

This general synthesis of 4-hydroxycoumarins can be represented by the following reactions in which R may be hydrogen, an alkyl, or an aryl residue

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(2) Campbell and Link, *J. Biol. Chem.*, **138**, 21 (1941).

(3) Stahmann, Huebner and Link, *ibid.*, **138**, 513 (1941).

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

(5) Overman, Stahmann, Sullivan, Huebner, Campbell and Link, *ibid.*, **142**, 941 (1942).

(6) For the possible therapeutic use of this drug in rendering blood less coagulable *in vivo* to control processes of embolism and thrombosis in man see the symposium in *J. Am. Med. Assoc.*, **120**, 1009–1026 (1942).

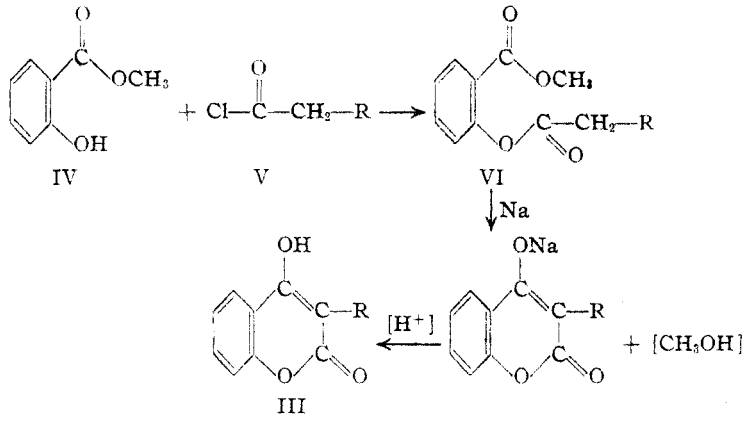
(7) Anschütz, *Ber.*, **36**, 465 (1903); *Ann.*, **367**, 169 (1909).

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

(9) Bauer and Schoder, *Arch. Pharm.*, **259**, 53 (1929).

(10) Hoesch, *Ber.*, **48**, 1122 (1915).

(11) Pauly and Lockemann, *ibid.*, **48**, 28 (1915).



As would be expected, many side reactions occur when these esters are treated with metallic sodium at the high temperature necessary for this condensation. These side reactions are major factors in limiting the yields of the 4-hydroxycoumarins. The crude reaction products were obtained as brown granular solids or heavy sirups which contained the sodium salt of the hydroxycoumarin. The 4-hydroxycoumarins were obtained from an aqueous solution of the crude salt by acidification. A dark oily or gummy non-volatile product usually separated from the aqueous solution at a *p*H above that at which the 4-hydroxycoumarins separated, and was removed at that point by extraction with an organic solvent. The volatile products formed during the condensation of methyl acetylsalicylate include salicylic acid, phenol, anisole, methyl acetate and acetic acid.

Table I lists the acyl derivatives of methyl salicylate which were condensed with sodium to form the 4-hydroxycoumarins presented in Table II.

The anticoagulant action of the 4-hydroxycoumarins reported here as well as many other coumarins has been under study since April, 1940,[12] and will be presented in a paper discussing the relation of structure to anticoagulant action. It should be noted that 3,3′-methylenebis-(4-hydroxycoumarin) (II) is a 3-substituted-4-hydroxycoumarin in which the substituent group contains another 4-hydroxycoumarin residue. 4-Hydroxycoumarin (I) and 4-hydroxycoumarins having an alkyl or aryl substituent group on carbon number 3 (III) also show anticoagulant action. The action of the lower members is slight but increases as the size of the substituent increases

(12) See sections in ref. 5, page 953, on Relation of Structure to Physiological Activity.

TABLE I

ESTERS OF METHYL SALICYLATE

Methyl ester	Yield, %	B. p., °C.	Mm.	Sp. gr., D^{25}	$n^{25}D$	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Acetylsalicylate[a]	95									
Propionylsalicylate	90	141–142	9	1.1579	1.5039	$C_{11}H_{12}O_4$	63.46	63.47	5.79	6.00
n-Butyrylsalicylate	81	155–156	12	1.1279	1.5011	$C_{12}H_{14}O_4$	64.86	64.85	6.30	6.42
Isobutyrylsalicylate	68	140–143	6	1.1467	1.5021	$C_{12}H_{14}O_4$	64.86	64.48	6.30	6.31
n-Valerylsalicylate	65	158–159	8	1.1014	1.4964	$C_{13}H_{16}O_4$	66.10	66.31	6.78	6.90
Isovalerylsalicylate	71	151–152	8	1.0980	1.4960	$C_{13}H_{16}O_4$	66.10	65.92	6.78	7.01
Capronylsalicylate	56	173–174	9	1.0874	1.4982	$C_{14}H_{18}O_4$	67.20	67.25	7.20	7.74
Heptoylsalicylate	73	181–182	9	1.0667	1.4941	$C_{15}H_{20}O_4$	68.18	68.31	7.50	7.86
Stearylsalicylate[b]	47	226–230	0.05			$C_{26}H_{42}O_4$	74.83	74.81	10.11	10.10
β-Phenylpropionylsalicylate	74	197–201	5	1.1768	1.5521	$C_{17}H_{16}O_4$	71.83	71.88	5.63	5.60
Phenylacetylsalicylate[c]	63									

[a] M. p. 47–49°. [b] M. p. 41–43°. [c] M. p. 59–60°. Pauly and Lockemann[11] report m. p. as 50°.

TABLE II

4-HYDROXYCOUMARINS

Compound	Yield, %	M. p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
4-Hydroxycoumarin[a]	22	214–216					
3-Methyl-4-hydroxycoumarin[b]	28	227–228					
3-Ethyl-4-hydroxycoumarin	28	155–156	$C_{11}H_{10}O_3$	69.47	69.57	5.26	5.41
3-*n*-Propyl-4-hydroxycoumarin	32	134–135	$C_{12}H_{12}O_3$	70.59	70.44	5.88	6.09
3-Isopropyl-4-hydroxycoumarin	25	172–174	$C_{12}H_{12}O_3$	70.59	71.15	5.88	6.01
3-Butyl-4-hydroxycoumarin	26	158–159	$C_{13}H_{14}O_3$	71.56	71.81	6.42	6.48
3-Amyl-4-hydroxycoumarin	30	137–139	$C_{14}H_{16}O_3$	72.41	72.22	6.90	6.91
3-Hexadecyl-4-hydroxycoumarin	21	96–97	$C_{25}H_{38}O_3$	77.72	77.35	9.85	9.77
3-Phenyl-4-hydroxycoumarin[c]	25	234–235					
3-Benzyl-4-hydroxycoumarin[d]	22	202–205					

[a] Anschütz[7] reports the m. p. as 204–206°. [b] Heilbron and Hill, *J. Chem. Soc.*, 1705 (1927), report the m. p. as 230°. [c] Pauly and Lockemann[11] report the m. p. as 236°. [d] Heilbron and Hill[b] report the m. p. as 205°.

and is greater for aryl than for alkyl groups.

Experimental

Preparation of Methyl Acetylsalicylate.—Methyl salicylate (2.0 kg.), acetic anhydride (2.0 kg.) and concentrated sulfuric acid (15 ml.) were mixed and allowed to stand at 40° in a cooling bath for forty minutes. The mixture was then slowly added to about 15 liters of cold water. The water contained a liberal amount of seed crystals from a previous batch and was well stirred during the addition. After standing six hours the crystalline ester was collected by filtration and washed thoroughly with water; yield 2430 g. (95%), m. p. 47–49°. The ester was used without recrystallization.

Preparation of 4-Hydroxycoumarin.—To a flask equipped with a stiff Hershberg stirrer, a thermometer well, a 12-inch Vigreux fractionating column with condenser, and an introduction port was added 1200 ml. of liquid paraffin (Stanolind) and 72 g. of sodium.[13] The flask was heated to 240° on a metal bath and 800 g. of methyl acetylsalicylate added over a period of thirty minutes. During this addition and for ninety minutes after the last portion of ester was added the reaction mixture was maintained at 240–250°. During the first part of the reaction 295 g. of distillate[14] was collected. The reaction mixture was filtered while hot and the brown granular solid cooled and washed with a low boiling petroleum fraction. The excess wash solvent was removed by drying and the solid slowly added to 3 liters of water at 65° with vigorous stirring. After thirty minutes the murky aqueous solution was slowly acidified at 50° to *p*H 5.5–6.0 with hydrochloric acid. During this acidification a precipitate of impurities separated. Between *p*H 5.5 and 6.0 the precipitate flocculated into a dark gummy mass which was skimmed off the top and discarded. The solution was cooled and extracted with an organic solvent, suitably ethyl ether, chloroform, or Skelly A to remove entrainments. The aqueous phase was then slowly acidified to *p*H 1.5. The crude 4-hydroxycoumarin crystallized during this acidification. After a few hours it was collected and recrystallized from 12 liters of boiling water. The yield was 145 g. (22%), m. p. 200–206°. Anschütz[7] reported the melting point as 204–206°. After repeated recrystallization the melting point was raised to 213–215°. About 4 g. of 3,3′-methylenebis-(4-hydroxycoumarin) were recovered from the mother liquors by the addition of formaldehyde.[3]

Because of the high temperature and large amount of sodium required in this condensation, it is most conveniently carried out on a larger scale in a cast iron reaction vessel. The synthesis has been carried out in a 2-gallon cast iron reaction kettle equipped with a mechanical stirrer and heated by a ring burner. Single 1600-g. lots of ester gave yields as good as those obtained from smaller runs. The use of cast iron equipment materially reduces the hazard of handling the large amounts of sodium at high temperatures and facilitates the temperature control.

Esterification of Methyl Salicylate.—Methyl propionylsalicylate was prepared by treating methyl salicylate with propionic anhydride by a procedure similar to that described for methyl acetylsalicylate. After decomposing the excess anhydride the remaining oil was separated, washed with sodium carbonate solution and distilled.

All other esters of methyl salicylate were prepared by refluxing equi-molar quantities of methyl salicylate and the corresponding acyl chloride until hydrochloric acid evolution was complete. These preparations were usually carried out with approximately 150 g. of methyl salicylate and hydrochloric acid evolution was then complete in from one to three hours. The reaction mixture was then distilled under reduced pressure.

Preparation of 3-Substituted 4-Hydroxycoumarins.—In a 500-ml. 3-neck, round-bottom flask equipped with a stiff Hershberg stirrer, reflux condenser and thermometer well, was placed 100 ml. of liquid paraffin (Stanolind) and an amount of sodium equivalent to 50 g. of the corresponding ester of methyl salicylate. The mixture was heated to 240–250° on a metal bath and 50 g. of ester slowly added through the condenser. The temperature was maintained at 240–250° until the reaction was complete as indicated by the disappearance of the sodium and the formation of the brown sodium salt which separated as a granular powder in the case of the lower esters or as a heavy sirup when derived from the higher esters. The reaction time was generally from forty to sixty minutes after all the ester had been added, and was determined by withdrawing samples and testing for unreacted sodium. When the reaction was complete, the paraffin was separated from the crude product by filtering or decanting. After cooling, the product was washed with a low boiling petroleum fraction, dried, and dissolved in 800 ml. of water. The murky solution was acidified with hydrochloric acid to the *p*H at which the 4-hydroxycoumarin just began to separate. The oily or gummy precipitate which had separated during the first part of the acidification was then removed by shaking with ethyl ether. The *p*H was between 6.5 and 7.5 for all 3-substituted-4-hydroxycoumarins except for 3-hexadecyl-4-hydroxycoumarin. The latter compound began to come out of solution above *p*H 9.0, so the alkaline aqueous solution was extracted with ethyl ether before the acidification. The aqueous phase was then acidified to *p*H 1.5. During this acidification the 3-substituted-4-hydroxycoumarins crystallized. In all cases except those noted in Table II, the crude products were recrystallized from ethanol–water mixtures.

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Summary

1. The preparation of 4-hydroxycoumarin from methyl acetylsalicylate by treatment with sodium has been studied. An improved method for preparing 4-hydroxycoumarin is presented.

2. The synthesis of nine 3-substituted-4-hydroxycoumarins from acylated derivatives of methyl salicylate is described.

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(13) The molar ratio of sodium to ester may be varied from about 0.6 to 1.0 without affecting the yield materially.

(14) This distillate contains about 22% salicylic acid, 11% phenol, 8% anisole, 6% acetic acid, 3% methyl acetate and other unidentified products.