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Coumarins. I. Derivatives of Coumarin-3- and 4-Carboxylic Acids

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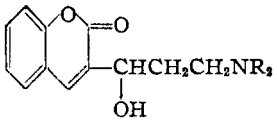
With the exception of a brief publication by Werder,[1] no pharmacological or chemical investigations appear to have been made of the basic esters and amides derivable from coumarins. Werder[1a,c] found that N,N-dialkyl-coumarin-3-carboxamides[2] exhibited remarkable sedative properties, and it is of considerable significance that they also had but slight toxicity. Furthermore, the favorable toxicity indices of coumarin-3-carboxylic acid were also apparent in salts of this acid with certain physiologically active bases such as ephedrine.[1b] These results are striking, since although coumarin itself has a slight narcotic activity, its toxic action is predominant.

In the same publication, Werder[1a] described 2-diethylaminoethyl coumarin-3-carboxylate hydrochloride and N-(2-diethylaminoethyl)-coumarin-3-carboxamide hydrochloride. The therapeutic properties of these compounds were not described; in particular no mention is made of observed local anesthetic activity. Local anesthetic activity in the coumarin-3-carboxylic acid esters and amides is of interest, since the compounds may be regarded as cinnamic acid types (*i. e.*, vinylogs of benzoic acid). In general, the basic esters of cinnamic acid show considerably greater activity than do the corresponding benzoates,[3] although toxicity has been found to increase proportionally.[4]

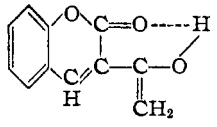
In the present work we have investigated a series of basic esters and amides derived from coumarin-3-carboxylic and coumarin-4-carboxylic acids. A subsequent communication will cover related compounds in the coumarin-3-acetic and coumarin-4-acetic acid series.

The substituted coumarin-3-carboxylic acids used in the present work were prepared by the conventional Knoevenagel method,[5] from a substituted salicylaldehyde and malonic ester. Modifications necessary in certain cases are detailed in the Experimental section. Only a single example of the coumarin-4-carboxylic acid type was investigated, due to the very low yields obtainable in the synthesis of these types.

An attempt was made to extend the investigation to the preparation of compounds of type I, through use of the Mannich reaction with 3-acetylcoumarin[6] followed by reduction (*e. g.*, by aluminum isopropylate). However, in spite of numerous experiments, under a variety of conditions, we were unable to isolate any reaction product when 3-acetylcoumarin was subjected to the Mannich reaction with diethylamine hydrochloride; in all cases only starting materials were recovered. This lack of reactivity may be due to stabilization of the hydrogen bonded structure II under the conditions of the Mannich reaction, for it is known that 3-acetylcoumarin readily forms an oxime[6] under normal conditions.



I



II

Werder[1c] prepared N,N-dimethylcoumarin-3-carboxamide from salicylaldehyde and malonic acid bis-dimethylamide at 145–150°. We were able to extend this method to the preparation of 2-diethylaminoethyl coumarin-3-carboxylate from salicylaldehyde and bis-(2-diethylaminoethyl) malonate, in high yield. It is interesting in this connection that reaction between the two components takes place only slowly at elevated temperature, even though tertiary amino groups are present, whereas the addition of catalytic amounts of piperidine or other secondary amine brought about an exothermic reaction at room temperature.

Among the coumarin-3-carboxylic acid derivatives prepared were a number of *bz*-nitro types. It was hoped that there could thus be prepared a series of *bz*-amino-substituted-coumarin-3-carboxylic acid derivatives, which would be vinylogs of aminobenzoic acid derivatives. The relative inaccessibility of 4-nitrosalicylaldehyde[7] precluded the preparation of 7-aminocoumarin derivatives, although the 6-nitro types are readily available.

In contrast to the easily manipulated reduction of 6-nitrocoumarin to 6-aminocoumarin either by iron–acetic acid[8] or electrolytically,[9] we were unable to prepare basic esters or amides of *bz*-aminocoumarin-3-carboxylic acids by either chemical or catalytic reduction. Part of the difficulty can be

(1) (a) Werder, *Merck Jahresberichte*, 88 (1936); (b) U. S. Patent 2,133,977; (c) U. S. Patent 2,170,127.

(2) Compounds of this type are indexed by *Chemical Abstracts* as 2-oxo-1,2-benzoylpyrone derivatives. For simplicity, we have used the conventional nomenclature.

(3) Gilman, *et al.*, THIS JOURNAL, **47**, 245 (1925); **50**, 437 (1928).

(4) *Cf.* also McElvain, *ibid.*, **49**, 2835 (1927); Bailey and McElvain, *ibid.*, **52**, 2007 (1930).

(5) Knoevenagel, *Ber.*, **31**, 2585 (1898).

(6) Prepared in 95% yield, m. p. 120–121°, by the method of Knoevenagel, *ibid.*, p. 732.

(7) Segesser and Calvin, THIS JOURNAL, **64**, 825 (1942).

(8) Clayton, *J. Chem. Soc.*, **97**, 1350 (1910); Morgan and Micklethwaite, *ibid.*, **85**, 1233 (1904).

(9) Kondo and Ui, *J. Pharm. Soc. Japan*, No. 498, 615 (1923).

ascribed to the ease with which the pyrone ring is opened in the presence of the new amino group or the basic group in the ester or amide linkage[10]; further, from the properties of the highly colored products which we have isolated from chemical reduction experiments it can be assumed that in this case reduction was incomplete. Catalytic reduction, with a variety of catalysts and under varying conditions, gave only the corresponding *bz*-amino dihydrocoumarins.[11] Judging from hydrogenation experiments there was no perceptible difference in the rate of reduction between the nitro group and the double bond.

Therapeutic assay of the coumarin-3-carboxylic acid derivatives for local anesthetic activity has been carried out by Drs. T. J. Becker and F. P. Luduena of these laboratories. A complete report will be published by these authors at a later date.

Experimental[12]

Salicylaldehydes.—The nitration of salicylaldehyde with fuming nitric acid (d. 1.49–1.50) in glacial acetic acid, and separation of the mixture of 3-nitro- and 5-nitrosalicylaldehydes, was carried out essentially by the method of Miller.[13] Bromination of the 3-nitro isomer according to the method of Auwers and Bürger,[14] or the nitration of 5-bromosalicylaldehyde,[14,15] gave high yields of 3-nitro-5-bromosalicylaldehyde. 2-Hydroxy-3-methoxy-5-nitrobenzaldehyde was prepared by the method of Dey and Kutti.[16] The bromination of 5-nitrosalicylaldehyde with bromine in glacial acetic acid solution at 30–40° gave a 70% yield of **3-bromo-5-nitrosalicylaldehyde,** long slender white needles from dilute acetic acid or from alcohol, m. p. 149–150°.

Anal. Calcd. for $C_7H_4BrNO_4$: C, 34.17; H, 1.64; N, 5.69. Found: C, 34.17; H, 1.87; N, 5.85.

The oxime formed pale yellow needles from dilute alcohol, m. p. 222.1–223.0°.

Anal. Calcd. for $C_7H_5BrN_2O_4$: N, 10.73. Found: N, 10.60.

The position of the bromine was proven by oxidation of the aldehyde to the known 3-bromo-5-nitrosalicylic acid with potassium permanganate in acetone. The product crystallized from 4% hydrochloric acid in long slender white needles, m. p. 225–226° (lit.,[17] m. p. 223–224° uncor.).

Ethyl Coumarin-3-carboxylates.—In general, the usual Knoevenagel procedure[5] was used to prepare the intermediate coumarin-3-carboxylates. However, in certain cases the salicylaldehydes (*e. g.*, 2-hydroxy-3-methoxy-5-nitrobenzaldehyde, 3-bromo-5-nitrosalicylaldehyde, etc.) were relatively slow in condensing by this method, and it was found necessary to force the reaction by heating and through use of increased amounts of the piperidine catalyst. A typical example follows:

A mixture of 25.0 g. (0.126 mole) of 2-hydroxy-3-methoxy-5-nitrobenzaldehyde, 25.0 g. (0.156 mole) of ethyl malonate, 30 ml. of absolute alcohol and 2 ml. of piperidine was refluxed for six hours. The orange colored reaction mixture was diluted with 400 ml. of alcohol, filtered, and the crystalline material was washed well with alcohol. Recrystallization from ethyl acetate gave 31.5 g. (85%) of ethyl 8-methoxy-6-nitrocoumarin-3-carboxylate as pale yellow needles.

The difficulty of preparing 2-hydroxy-3-methoxy-6-nitrobenzaldehyde[16] precluded the possibility of preparing 8-methoxy-5-nitrocoumarin-3-carboxylic acid in this manner. Dey and Kutti[16] have reported that the nitration of 8-methoxycoumarin-3-carboxylic acid takes place exclusively in the 5-position; in our hands, however, the method did not prove adaptable to large-scale preparations. We have found that nitration of the ethyl ester gives fair results, although the yields are low:

To 100 ml. of concentrated nitric acid (d. 1.42) at 25° was slowly added 24.8 g. (0.10 mole) of ethyl 8-methoxycoumarin-3-carboxylate[18] with stirring. The resulting solution was slowly heated to 40–45° and maintained at this temperature (the reaction becomes uncontrollable above 55°) by alternate cooling and heating for one hour. The solution was poured into one liter of ice-water with stirring, and the resulting precipitate was filtered and washed thoroughly with water. After two recrystallizations of the air-dried material from ethyl acetate with decolorization there was obtained a 22% yield of ethyl 8-methoxy-5-nitrocoumarin-3-carboxylate as pale yellow needles, m. p. 184–186°.

Anal. Calcd. for $C_{13}H_{11}NO_7$: N, 4.78. Found: N, 4.75.

Saponification of the ethyl ester gave a 92% yield of 8-methoxy-5-nitrocoumarin-3-carboxylic acid, m. p. 215–217° (lit.,[16] m. p. 203° uncor.).

Anal. Calcd. for $C_{11}H_7NO_7$: C, 49.81; H, 2.64. Found: C, 50.04; H, 2.73.

Coumarin-3-carboxylic Acids.—The ethyl esters were saponified by refluxing with an excess of dilute sodium hydroxide solution for several hours, followed by acidification with hydrochloric acid. It was generally preferable to pour the solution of the sodium salt into an excess of strong hot hydrochloric acid, since by the reverse process there was sometimes obtained a substantial amount of the coumaric acid.

Coumarin-3-carbonyl Chlorides.—The acid chlorides were prepared by the action of thionyl chloride on the acid, without solvent[19]:

A mixture of 20 g. (0.075 mole) of 8-methoxy-6-nitrocoumarin-3-carboxylic acid and 119 g. (1.0 mole) of pure thionyl chloride[20] was refluxed under anhydrous conditions for two hours. Solution was complete after twenty minutes. The excess thionyl chloride was distilled under reduced pressure, and the crystalline residue was taken down twice with 250-ml. portions of dry benzene. Recrystallization of the residual solid from dry benzene gave 21.2 g. (97%) of 8-methoxy-6-nitrocoumarin-3-carbonyl chloride.

Coumarin-3-carboxylates, -thiolcarboxylates and -carboxamides.—The coumarin-3-carboxylates were prepared by several methods. Reaction between the basic alcohol and a coumarin-3-carbonyl chloride in dry benzene gave high yields, as did the reaction between a coumarin-3-carboxylic acid and an ω-dialkylaminoalkyl halide in iso-

(10) Both Clayton and Morgan, ref. 8, have pointed out the intense yellow and orange colors of the pure *bz*-aminocoumarins. On structural grounds it is apparent from these observations and from the observed high melting points that the *bz*-aminocoumarins must exist at least partially in the open (cinnamic acid salt) forms.

(11) *Cf.* Smith and Byers, THIS JOURNAL, **63**, 612 (1941).

(12) All melting and boiling points are corrected. The authors are indebted to Mr. Morris E. Auerbach and staff for the analyses.

(13) Miller, *Ber.*, **20**, 1927 (1887).

(14) Auwers and Bürger, *ibid.*, **37**, 3934 (1904).

(15) Auwers and Walker, *ibid.*, **31**, 3037 (1898); Raiford and Tanzer, *J. Org. Chem.*, **6**, 730 (1941). The latter authors do not give preparation details nor yields. In the present work the compound was prepared by the addition, during four hours, of two moles of bromine to two moles of salicylaldehyde (each dissolved in four volumes of chloroform) with stirring, at 30°. The mixture was then refluxed for two hours, allowed to stand overnight, and the chloroform removed *in vacuo*. Crystallization of the residue from Skellysolve C gave an 84% yield of white product, m. p. 105–106°.

(16) Dey and Kutti, *Proc. Nat. Inst. Sci., India*, **6**, 641 (1940); *cf.* Davies, *J. Chem. Soc.*, **123**, 1575 (1923).

(17) Lallmann and Grothmann, *Ber.*, **17**, 2724 (1884); Chattaway and Goepp, *J. Chem. Soc.*, 699 (1933).

(18) Perkin and Robinson, *J. Chem. Soc.*, **105**, 2382 (1914).

(19) *Cf.* Boehm and Schumann, *Arch. Pharm.*, **271**, 490 (1933).

(20) The high ratio of thionyl chloride to acid was used to increase the rate of reaction through increased solubility.

propyl alcohol.[21] A third method consisted of the transesterification of the ethyl coumarin-3-carboxylate with a basic alcohol, in certain cases using toluene as a diluent. The yields by this latter method were quite good, but purification of the product from traces of starting materials often proved difficult. A further method was the direct synthesis from suitable malonic esters, although this method gave low over-all yields because of the difficulty of preparing the required basic esters of malonic acid:

A mixture of 160 g. (1.0 mole) of redistilled diethyl malonate, 250 g. (2.14 moles) of redistilled 2-diethylaminoethanol and 400 ml. of dry toluene was distilled slowly during eight hours through a 14" vacuum-jacketed Vigreux column surmounted by a total reflux, variable takeoff distillation head. A total of 435 ml. of distillate was collected, and the final internal temperature was 152°. Fractionation of the pale yellow-colored still residue gave 95.2 g. of a colorless liquid, b. p. 91–102° at 0.2–0.5 mm. (with slight decomposition).[22]

A mixture of 12.2 g. of salicylaldehyde and 30.2 g. of the above crude bis-(2-diethylaminoethyl) malonate gave no evidence of reaction, either when heated at 100° or when treated with, *e. g.*, pyridine. However, the addition of 10 drops of piperidine brought about an immediate coloration and an exothermic reaction. After heating on the steam-bath at 100° for three hours, the product was recrystallized from alcohol and converted to the hydrochloride with alcoholic hydrogen chloride. The yield of 2-diethylaminoethyl coumarin-3-carboxylate hydrochloride, m. p. 211–212°,[1a] was excellent.

The thiolcarboxylates were prepared by the direct reaction between a coumarin-3-carboxyl chloride and an ω-dialkylaminoalkanethiol in dry benzene.[23] The yields were essentially quantitative, and the products were easily purified.

The preparation of the coumarin-3-carboxamides was most conveniently carried out by direct interaction between a coumarin-3-carbonyl chloride and the dialkylaminoalkylamine in cold dry benzene. In certain cases (*e. g.*, with 4-diethylamino-1-methylbutylamine) a purer product was obtained by amination in a mixture of chloroform, water and sodium bicarbonate.[24] Amination by reaction between the ethyl ester and an amine gave unworkable mixtures (ring opening was very extensive under these conditions).

The properties and yields of the coumarin-3-carboxylates, -thiolcarboxylates, -carboxamides, and intermediates in their preparation, are given in Table I.

7-Hydroxycoumarin-4-carboxylic Acid.—The method of v. Pechman and Graeger[25] was modified through substitution of commercial sodio-oxalacetic ester for the oxalacetic ester–sodium ethylate mixture used by these workers. The yield (on one-mole runs) was 46%. Saponification of the ethyl ester according to v. Pechman and Graeger[25,26] gave 98% yields of purified acid, m. p. 245–246° (reported,[25] m. p. 247–248° uncor.).

2-Diethylaminoethyl 7-Hydroxycoumarin-4-carboxylate Hydrochloride.—A mixture of 5 g. of 7-hydroxycoumarin-4-carboxylic acid, 3 g. of 2-diethylaminoethyl chloride and 50 ml. of isopropyl alcohol was refluxed for one hour. A yellow crystalline precipitate appeared after twenty minutes. The reaction mixture was cooled, filtered, and the product was washed with cold isopropyl alcohol. Two recrystallizations from a large volume of ethanol gave 7.0 g. of long slender yellow needles, m. p. 192.7–193.9°.

Anal. Calcd. for $C_{16}H_{20}ClNO_5$: C, 56.22; H, 5.90; Cl, 10.38. Found: C, 56.31; H, 5.65; Cl, 10.30.

The following compounds were prepared by a similar method:

2-(1-Piperidyl)-ethyl 7-hydroxycoumarin-4-carboxylate hydrochloride, yellow leaflets from hot water, m. p. 213.4–215.0°.

Anal. Calcd. for $C_{17}H_{20}ClNO_5$: C, 57.72; H, 5.70; N, 3.96; Cl, 10.02. Found: C, 57.81; H, 5.49; N, 3.92; Cl, 9.90.

3-(4-Morpholinyl)-propyl 7-hydroxycoumarin-4-carboxylate hydrochloride, pale yellow prisms from dilute hydrochloric acid, m. p. 233.0–233.8°.

Anal. Calcd. for $C_{17}H_{20}ClNO_6$: C, 55.21; H, 5.45; N, 3.79; Cl, 9.59. Found: C, 55.44; H, 5.46; N, 3.67; Cl, 9.53.

The reaction failed with 2-dimethylaminoethyl chloride. The trans-esterification of ethyl 7-hydroxycoumarin-4-carboxylate by means of a basic alcohol in toluene gave good results, although purification proved difficult. Attempts to aminate the ethyl ester by means of an ω-dialkylaminoalkylamine failed.

The Reduction of Nitrocoumarin-3-carboxylic Acid Derivatives.—Both chemical and catalytic reductions were tried as means of securing *bz*-aminocoumarin-3-carboxylic acid derivatives; both methods failed.

Catalytic Reductions.—Hydrogenation of the nitrocoumarin derivatives was carried out in a modified[27] Parr–Burgess apparatus. No difference was observed in products obtained when the catalyst was varied between platinum, palladium or Raney nickel. Plots of hydrogen uptake *versus* time showed no definite change in slope ascribable to a difference in the rates of reduction between a nitro group and a double bond. A typical reduction follows:

A solution of 3.0 g. of ethyl 6-nitrocoumarin-3-carboxylate in 100 ml. of ethyl acetate was mixed with 5 g. of neutral Raney nickel catalyst and shaken with hydrogen at 2.5–3 atmospheres. Only a slow hydrogen uptake was evident at room temperature (ninety-six hours for completion) but reduction was initially rapid at 50°. During the latter stages of the reduction an orange precipitate appeared in the mixture (*vide infra*), and the reduction rate slowed while this precipitate gradually redissolved. At completion of the reduction (twelve to forty-eight hours at 50°) the solution was clear and colorless. After filtration of catalyst, the filtrate was diluted with a large volume of Skellysolve A. The resulting precipitate was filtered and recrystallized from a warm ethyl acetate–Skellysolve A mixture. **Ethyl 6-amino-3,4-dihydrocoumarin-3-carboxylate** crystallized in clusters of white cottony needles, m. p. 90–91° (dec.). The yield was 90%.

Anal. Calcd. for $C_{12}H_{13}NO_4$: C, 61.23; H, 5.53; N, 5.96. Found: C, 61.33; H, 5.45; N, 6.02.

The hydrochloride precipitated as white needles when an ethyl acetate solution of the base was treated with ethereal hydrogen chloride, m. p. 211–212° (dec.).

Anal. Calcd. for $C_{12}H_{14}ClNO_4$: C, 53.04; H, 5.16; N, 5.16. Found: C, 53.08; H, 4.96; N, 5.10.

The use of platinum or palladium catalysts increased the rate of reduction but did not affect the phenomena observed. In a manner similar to the above, there were prepared:

Ethyl 5-amino-8-methoxy-3,4-dihydrocoumarin-3-carboxylate, *via* an intermediate insoluble red-brown precipitate (*vide infra*); cottony white needles from ethyl acetate–Skellysolve A, m. p. 126–128°, yield 92%.

Anal. Calcd. for $C_{13}H_{15}NO_5$: C, 58.87; H, 5.66; N, 5.28. Found: C, 58.52; H, 5.64; N, 5.22.

The hydrochloride crystallized from absolute alcohol–ethyl acetate in white needles, m. p. 185–186°.

Anal. Calcd. for $C_{13}H_{16}ClNO_5$: N, 4.64. Found: N, 4.56.

(21) Hörenstein and Pählicke, *Ber.*, **71**, 1644 (1938). In the special case of nitrosubstituted coumarin-3-carboxylic acids the yields were lowered.

(22) Gilman and Johnson, THIS JOURNAL, **50**, 3346 (1928), prepared the compound from malonyl chloride and 2-diethylaminoethanol. They record a b. p. of 163° at 4.5 mm.

(23) Clinton, Salvador and Laskowski, *ibid.*, **71**, 3366 (1949).

(24) Clinton, Salvador, Laskowski and Suter, *ibid.*, **70**, 950 (1948).

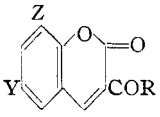
(25) v. Pechman and Graeger, *Ber.*, **34**, 378 (1901).

(26) *Cf.* Dey, *J. Chem. Soc.*, **107**, 1606 (1915).

(27) Buck and Jenkins, THIS JOURNAL, **51**, 2163 (1929).

TABLE I

COUMARIN-3-CARBOXYLIC ACID DERIVATIVES



Y	Z	R	M. p., °C.	Yield, %	Analyses, % Calcd.	Found	Calcd.	Found
H	H	$-OCH_2CH_3$[a]	95–96	77	C, 66.06	66.03	H, 4.59	4.69
H	H	$-OH$[b]	191–192	96	C, 63.16	63.26	H, 3.16	3.38
H	H	$-Cl$[c]	147–148	78				
H	H	$-OCH_2CH_2N(C_2H_5)_2\cdot HCl$[d]	211–212	75				
H	H	$-OCH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot HCl$	125–126	76	Cl, 9.66	9.52	N, 3.81	3.79
H	H	$-SCH_2CH_2N(C_2H_5)_2\cdot HCl$	189–191	77	S, 9.37	9.11	N, 4.10	4.13
H	H	$-OCH_2CH_2SCH_2CH_2CH_2NC_5H_{10}$[e]$\cdot HCl$	118–119	69	S, 7.78	7.79	N, 3.40	3.36
H	H	$-N(C_2H_5)_2$[d]	77–78	80	N, 5.71	5.77		
H	H	$-NHCH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot HCl$[f]	141–144	70	Cl, 9.69	9.89	N, 7.64	7.63
NO_2	OCH_3	$-OCH_2CH_3$[g]	210–210.5	85	C, 53.25	53.20	H, 3.75	3.52
NO_2	OCH_3	$-OH$[h]	219–220	99	C, 49.81	49.76	H, 2.64	2.83
NO_2	OCH_3	$-Cl$	179–180	85	Cl, 12.52	12.60		
NO_2	OCH_3	$-OCH_2CH_2N(C_2H_5)_2\cdot HCl$	191–192	90	Cl, 8.86	8.80	N, 6.99	6.83
NO_2	OCH_3	$-N(C_2H_5)_2$	192–193	93	N, 8.75	8.68		
H	OCH_3	$-OCH_2CH_3$[i]	95–96	81				
H	OCH_3	$-OH$[i]	212–213	99	C, 60.00	60.00	H, 3.64	3.73
H	OCH_3	$-Cl$	171–172	99	Cl, 14.88	14.91		
H	OCH_3	$-OCH_2CH_2N(C_2H_5)_2\cdot HCl$	195–196	75	Cl, 9.99	9.99	N, 3.94	3.74
H	OCH_3	$-N(C_2H_5)_2$	107–108	51	N, 5.09	4.90		
H	NO_2	$-OCH_2CH_3$[j]	160–161	40	C, 54.75	54.63	H, 3.42	3.22
H	NO_2	$-OH$[k]	191–192	90	C, 51.06	51.12	H, 2.12	2.27
NO_2	H	$-OCH_2CH_3$[l]	200–201	66				
NO_2	H	$-OH$[l]	235–236	97	N, 5.96	5.99		
NO_2	H	$-Cl$[l]	172–173	90				
NO_2	H	$-OCH_2CH_2N(C_2H_5)_2\cdot HCl$	197–198	90	N, 7.56	7.65		
NO_2	H	$-N(C_2H_5)_2$[m]	184–185	72	C, 57.93	58.04	H, 4.83	4.86
NO_2	Br	$-OCH_2CH_3$	184–185	10	N, 4.09	4.10		
NO_2	Br	$-OH$	221–222	40	Br, 25.47	25.42	N, 4.46	4.69
Br	H	$-OCH_2CH_3$[n]	168–169	88	C, 48.48	48.24	H, 3.03	3.00
Br	H	$-OH$[o]	199	99	Br, 29.74	29.55		
Br	H	$-Cl$	160–161	94	Cl, 12.35	12.30		
Br	H	$-OCH_2CH_2N(C_2H_5)_2\cdot HCl$	193–194	89	Cl, 8.78	8.94	N, 3.46	3.53
Br	H	$-N(C_2H_5)_2$	160–161	71	Br, 24.69	24.27	N, 4.32	4.36
Br	H	$-NHCH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot HCl$	170–172	75	Cl, 7.97	7.96	N, 6.29	6.08
Br	H	$-OCH_2CH_2SCH_2CH_2N(C_2H_5)_2\cdot HCl$	166–167	75	S, 6.89	6.65	N, 3.01	3.01
Br	H	$-SCH_2CH_2CH_2CH_2N(C_2H_5)_2\cdot HCl$	209–210	80	Cl, 7.92	8.02		
Br	H	$-SCH_2CH_2N(C_2H_5)_2\cdot HCl$	210–212	37	S, 7.61	7.34	N, 3.33	2.39
Br	H	$-SCH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot HCl$	111–113	65	S, 6.92	6.61	N, 3.03	2.90

[a] Baker and Lapworth, *J. Chem. Soc.*, **127**, 566 (1925). [b] Knoevenagel, *Ber.*, **31**, 2618 (1898). [c] Boehm and Schumann, *Arch. Pharm.*, **271**, 490 (1933), report m. p. 147–148°; the m. p. of 136–137° reported by Lampe and Trenknerowna, *Roczniki Chem.*, **14**, 1231 (1934), is apparently in error. [d] Werder, *Merck Jahresb.*, 88 (1936). [e] $-NC_5H_{10}$ is 1-piperidyl. [f] This compound was also prepared as a salt of coumarin-3-carboxylic acid: needles from methanol–ether, m. p. 156–157°. Calcd. for $C_{19}H_{26}N_2O_3\cdot C_{10}H_6O_4$: N, 5.38. Found: N, 5.31. [g] Calcd. for $C_{13}H_{11}NO_7$: N, 4.78. Found: N, 4.76. [h] Calcd. for $C_{11}H_7NO_7$: N, 5.28. Found: N, 5.39. [i] Perkin and Robinson, *J. Chem. Soc.*, **105**, 2382 (1914). [j] Calcd. for $C_{12}H_9NO_6$: N, 5.32. Found: N, 5.38. [k] Calcd. for $C_{10}H_5NO_6$: N, 5.96. Found: N, 5.96. [l] Lampe and Macierewicz, *Roczniki Chem.*, **18**, 668 (1938). [m] Calcd. for $C_{14}H_{14}N_2O_5$: N, 9.66. Found: N, 9.66. [n] Calcd. for $C_{12}H_9BrO_4$: Br, 26.94. Found: Br, 26.70. [o] Pandya and Pandya, *Proc. Indian Acad. Sci.*, **18A**, 164 (1943).

Ethyl 6-amino-8-methoxy-3,4-dihydrocoumarin-3-carboxylate, *via* an intermediate insoluble red-orange precipitate (*vide infra*); rosets of white needles from ethyl acetate–Skellysolve B, m. p. 81–82°, yield 93%.

Anal. Calcd. for $C_{13}H_{15}NO_5$: N, 5.28. Found: N, 5.32.

The hydrochloride formed white needles from absolute alcohol–ether, m. p. 185–190° (dec.).

Anal. Calcd. for $C_{13}H_{16}ClNO_5$: N, 4.64; Cl, 11.77. Found: N, 4.66; Cl, 11.65.

In solution the 3,4-dihydrocoumarin-3-carboxylates were very unstable to heat and to air. This phenomenon has been observed in a similar case by Smith and Byers.[11]

Chemical Reductions.—Application of the iron-acetic acid method of Clayton[8] to ethyl 8-methoxy-6-nitrocoumarin-3-carboxylate gave no reduction. Use of the general reduction procedure of West[28] afforded a high yield of a product which crystallized in red-orange needles from dilute alcohol, m. p. 149–150°. This material, of unknown constitution, proved to be identical (mixed

(28) West, *J. Chem. Soc.*, **127**, 494 (1925).

m. p.) with the intermediate colored insoluble reduction product observed during the catalytic reduction of ethyl 8-methoxy-6-nitrocoumarin-3-carboxylate (*vide supra*).

Anal. Found: C, 57.75; H, 4.98; N, 5.95.

A similar reduction of 2-diethylaminoethyl 8-methoxy-6-nitrocoumarin-3-carboxylate hydrochloride gave, as the only isolatable reduction product, a solid crystallizing from absolute alcohol–Skellysolve A in white needles, m. p. 134–135°.

Anal. Found: C, 46.06; H, 9.09.

The reduction of ethyl 6-nitrocoumarin-3-carboxylate by West's method gave a brown solid, crystallizing from dilute alcohol in bright yellow needles, m. p. 161–163°. This solid was identical (mixed m. p.) with the intermediate insoluble precipitate observed during catalytic reduction of the same compound (*vide supra*).

Anal. Found: C, 58.47; H, 4.73.

Similarly, the chemical reduction of N,N-diethyl-6-nitrocoumarin-3-carboxamide gave a bright yellow unstable solid, m. p. 110–115° (from benzene–Skellysolve A).

Anal. Found: C, 68.97; H, 5.83; N, 10.41.

Summary

There has been described the preparation of a series of substituted coumarin-3- and coumarin-4-carboxylic acid derivatives. Attempts to prepare *bz*-amino members of this series resulted either in 3,4-dihydrocoumarins or in highly colored compounds of unknown constitution.

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[Contribution from the Eastern Regional Research Laboratory[1]]

A Crystalline Compound of β-Lactoglobulin with Dodecyl Sulfate[2]

By T. L. McMeekin, B. D. Polis, E. S. DellaMonica and J. H. Custer

Previous studies[3,4,5,6] have shown that proteins are precipitated from solution by synthetic detergents. Anionic detergents such as dodecyl sulfate precipitate proteins from acid solutions, whereas cationic detergents precipitate proteins from alkaline solutions. In the *p*H region close to the isoelectric point, neither cationic nor anionic detergents form precipitates with proteins.[6] Precipitated protein detergent complexes are soluble in an excess of detergent, accompanied by the denaturation of the protein and the liberation of free sulfhydryl groups.[3] The interaction of proteins with synthetic detergents has been extensively reviewed by Putnam.[7]

The work reported here deals with the preparation and properties of a crystalline complex of β-lactoglobulin combined with small quantities of dodecyl sulfate.

Experimental

Preparation of β-Lactoglobulin.—Crystalline β-lactoglobulin was prepared from unpasteurized milk by the method of Palmer.[8] The β-lactoglobulin contained 15.6% nitrogen and was electrophoretically homogeneous at *p*H 8.4 but inhomogeneous at *p*H 4.7, as was demonstrated by Li.[9]

Purified sodium dodecyl sulfate was used. A 0.01 molar aqueous solution, made to *p*H 4.2 with acetic acid, was used in precipitating β-lactoglobulin.

Preparation of Crystalline β-Lactoglobulin-dodecyl Sulfate.—β-Lactoglobulin–dodecyl sulfate was prepared by several procedures, in which the proportion of dodecyl sulfate to protein ranged from 4.2 to 14.0 cc. of 0.01 molar dodecyl sulfate per gram of protein. In every case, the crystalline protein prepared by dialysis at *p*H 5.1–5.2 after the removal of dodecyl sulfate with barium chloride, as described by Putnam and Neurath,[6] differed from β-lactoglobulin in solubility and mobility. Figure 1 gives a comparison of normal and dodecyl sulfate β-lactoglobulin electrophoretic patterns and mobilities in acetate buffer at *p*H 4.8 and veronal buffer at *p*H 8.4.

By adding 4.4 cc. of 0.01 *M* dodecyl sulfate to 50 cc. of a 2.1% solution of protein at *p*H 4.8, it was possible to crystallize the modified β-lactoglobulin directly, without the preliminary formation of a precipitate or the use of barium chloride to remove dodecyl sulfate. On standing for several hours, characteristic crystals appeared which had the electrophoretic mobility of modified β-lactoglobulin, demonstrating that only a small amount of dodecyl sulfate is necessary to modify the properties of β-lactoglobulin and that barium ions or barium sulfate do not produce the modification. The preparation which has been analyzed most completely was made as follows: approximately 10 g. of crystalline β-lactoglobulin suspended in 250 cc. of water was dissolved in dilute acetic acid and made to *p*H 4.2. Then 70 cc. of 0.1 *N* dodecyl sulfate at *p*H 4.2 was added with stirring. The small amount of precipitate formed was ignored. The solution then was made to *p*H 6.0 by adding dilute ammonia. The excess dodecyl sulfate was precipitated by adding 5 cc. of a 5% solution of barium chloride. After thirty minutes, the precipitated barium dodecyl sulfate was removed by centrifugation, and the supernatant was adjusted to *p*H 5.1. On dialysis, a yield of about 8 g. of crystalline protein was obtained. This material had a mobility u(sq. cm. volt^{-1} sec.$^{-1}$ $\times 10^5$) of 1 $u \times 10^5$ at *p*H 4.7 in acetate buffer and a mobility of $-5.9\ u \times 10^5$ at *p*H 8.4 in veronal buffer of 0.1 ionic strength. After several recrystallizations of the protein by dialysis from salt solutions, the properties were unchanged.

Table I shows the total nitrogen, α-amino nitrogen and total sulfur contents.

Properties of β-Lactoglobulin Dodecyl Sulfate

The analytical data in Table I indicate that two molecules of dodecyl sulfate are bound to one molecule of β-lactoglobulin. The method of preparation involving dialysis and treatment with barium to remove the insoluble barium salt of do-

(1) One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Philadelphia. Article not copyrighted.

(2) A preliminary report of this work was presented at the meeting of the American Society of Biological Chemists, Atlantic City, March, 1948; *Federation Proc.*, **7**, 172 (1948).

(3) Anson, *J. Gen. Physiol.*, **23**, 239 (1939).

(4) McMeekin, *Federation Proc.*, **1**, 125 (1942).

(5) Putnam and Neurath, *J. Biol. Chem.*, **150**, 263 (1943).

(6) Putnam and Neurath, This Journal, **66**, 692 (1944).

(7) Putnam, "Advances in Protein Chemistry," Vol. IV, Academic Press, Inc., New York, N. Y., 1948, p. 79.

(8) Palmer, *J. Biol. Chem.*, **104**, 359 (1934).

(9) Li, This Journal, **68**, 2746 (1946).