ether. After the reaction mixture had been stirred for three minutes at room temperature, 40 ml. of dry chloroform containing 17.4 g. (0.124 mole) of hexamethylenetetramine was added with stirring. Thus all chloromethyl compounds including unchanged chloromethyl ether were precipitated as the salt. The mixture was stirred for three hours before removing the salts. The mixture of salts was dissolved in 100 ml. of water and heated under reflux for four hours. After filtering while hot and then cooling, light yellow needles separated. These were recrystallized from hot water; yield one-half gram, m. p. 204°. *Anal.* Calcd. for $C_{10}H_{10}O_4$: C, 61.84; H, 5.19. Found: C, 61.98; H, 5.28.

Alkaline oxidation of the dialdehyde by 5% potassium permanganate gave the known 4,6-dimethoxyisophthalic acid.

2,5-Dimethoxyterephthalaldehyde.—A solution of 257.5 g. (0.5 mole) of the salt of 2,5-bis-(chloromethyl)-hydroquinone dimethyl ether[9] in 2 l. of water containing 100 ml. of 40% formalin was refluxed for four hours with stirring. A yellow precipitate formed and was filtered off after the mixture had cooled to room temperature. This yellow solid, 2,5-dimethoxyterephthalaldehyde, was converted into the bisulfite salt by heating with an excess of sodium metabisulfite dissolved in about 2 l. of water. As soon as solution of the aldehyde was complete, the hot solution was filtered to remove a small amount of insoluble material. After heating the filtrate for about an hour, concentrated hydrochloric acid was added slowly and with stirring. Care had to be taken while adding the acid in order that the sulfur dioxide evolved did not cause the mixture to foam over the sides of the beaker; vigorous stirring helped prevent excessive foaming. After adding enough hydrochloric acid to give about a 25% excess, the mixture was left standing three hours on the steam plate. The bright yellow solid that had settled out was filtered off and pressed as dry as possible on the funnel. It was washed by transferring to a 1500-ml. beaker and stirring with 1 liter of distilled water. The water was removed by filtration. The washing process was repeated twice. Finally, the material was filtered, washed with methanol, and dried in a vacuum desiccator over calcium chloride. The bright-yellow 2,5-dimethoxyterephthalaldehyde obtained weighed 61 g. (63%) and melted at 206°. A small sample recrystallized from 95% ethanol melted at 207°. *Anal.* Calcd. for $C_{10}H_{10}O_4$: C, 61.84; H, 5.19. Found: C, 61.43; H, 5.41.

2,5-Dimethylterephthalaldehyde.—To a solution of 5 g. (0.025 mole) of 2,5-bis-(chloromethyl)-*p*-xylene in 100 ml. of 60% alcohol was added 7 g. (0.05 mole) of hexamethylenetetramine. This mixture was then refluxed for eighteen hours. On cooling, 1.84 g. of crude dialdehyde precipitated. One recrystallization from 60% alcohol gave 1.5 g. (38%) of pure 2,5-dimethylterephthalaldehyde, m. p. 102–103°. *Anal.* Calcd. for $C_{10}H_{10}O_2$: C, 74.07; H, 6.17. Found: C, 74.45; H, 6.15.

The **phenylhydrazone** was prepared in usual way, m. p. 232°. *Anal.* Calcd. for $C_{22}H_{22}N_4$: N, 16.37. Found: N, 16.29.

Abnormal Sommelet Reactions.—Bis-(chloromethyl)-mesitylene, *o*-xylylene bromide, 4,5-bis-(chloromethyl)-xylene,[8] 4,5-bis-(chloromethyl)-veratrole[8] and 3,4-bis-(chloromethyl)-veratrole[8] readily formed the hexamethylenetetramine salts in quantitative yields. Neutral hydrolysis of the salts gave only basic nitrogen compounds. These basic nitrogen compounds are being further investigated.

(9) The chloromethyl compound was made by the method of Wood and Gibson, THIS JOURNAL, **71**, 393 (1949).

Summary

1. The reaction of ten bis-(halogenomethyl)-benzenes with hexamethylenetetramine in chloroform solutions is described.

2. Hydrolysis of the salts of *m*- and *p*-bis-(chloromethyl)-benzenes according to Sommelet's procedure gave satisfactory yields of *m*- and *p*-dialdehydes except in the cases where the two nuclear carbons *ortho* to each chloromethyl group were substituted. These formed basic nitrogen compounds instead.

3. Hydrolysis of the salts of *o*-bis-(chloromethyl)-benzenes gave basic nitrogen compounds rather than *o*-dialdehydes.

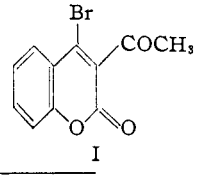
KNOXVILLE, TENN. RECEIVED NOVEMBER 1, 1949

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

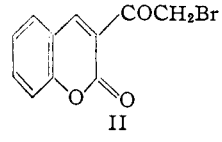
Bromination of 3-Acetocoumarin

BY C. F. KOELSCH

When 3-acetocoumarin is treated with bromine, there is obtained a monobromo derivative to which structure I has been assigned.[1] Such a derivative would be a useful intermediate in syntheses of compounds related to morphine. But the evidence for structure I, formation of an unstable addition compound during the bromination, and formation of salicylic acid when the bromo-compound is fused with alkali, does not exclude structure II. A study of the behavior of the substance toward thiourea now indicates that structure II is correct.



I

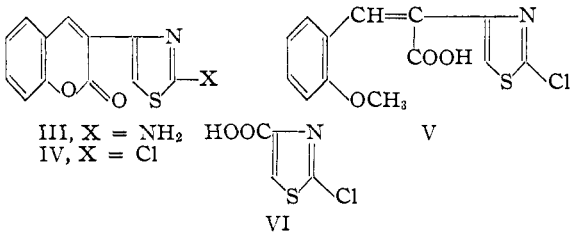


II

(1) Rap, *Gazz. chim. ital.*, **27**, II, 500 (1897).

Of the compounds corresponding to structures I and II, only the latter could yield a thiazole with thiourea, and degradation shows that a thiazole, III, is formed with this reagent. When the thiourea product is diazotized in strong hydrochloric acid, it yields a chloro compound, IV. With alkali and methyl sulfate, the chloro compound yields an acid, V, and when this acid is oxidized with permanganate, it furnishes *o*-methoxybenzaldehyde and a new acid, VI. The composition of the latter indicates that it must contain a thiazole nucleus.

Because the bromination product from 3-acetocoumarin is not the nuclear derivative, I, it cannot be used as originally planned. But a preliminary investigation has shown that its

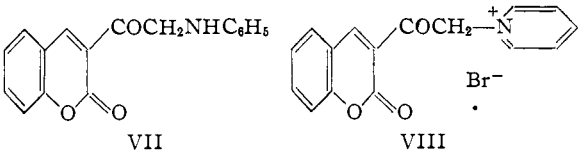


III, X = NH_2
IV, X = Cl

V

VI

reactions with basic reagents are worth further study. Strong bases, sodium methoxide and piperidine, yield black tars. Aniline yields a crystalline compound which may be VII, but the right red color of the compound indicates that this structure must be used tentatively. Pyridine and similar heterocyclic bases yield addition products which may be simply quaternary salts, VIII, but again these structures require further investigation, for the materials obtained from the salts with aqueous alkali are remarkably water-insoluble and base-stable.[2]



VII

VIII

Experimental

3-(ω-Bromoacetyl)-coumarin, II.—To a solution of 47 g. of 3-acetylcoumarin in 200 ml. of alcohol-free chloroform was added 40 g. of bromine in 25 ml. of chloroform, with intermittent shaking and warming to decompose an addition product. The mixture was heated for fifteen minutes on a water-bath to expel most of the hydrogen bromide, then cooled and filtered. The solid was washed with ether, giving 51–59 g. of nearly pure product; crystallization from acetic acid gave nearly colorless needles, m. p. 163–165°, that did not decompose at 175° (literature[1] m. p. 162°, dec. 166°).

2-Amino-4-(3-coumarinyl)-thiazole, III.—When a suspension of 2.7 g. of II in 15 ml. of hot alcohol was treated with 1.6 g. of thiourea, a smooth exothermic reaction took place, giving a clear solution that soon deposited crystals. The deposit was removed, washed with alcohol, and then boiled with water containing sodium acetate. This furnished 2.2 g. of bright yellow needles, m. p. 220–225°; crystallization from 200 ml. of alcohol gave a pure product, m. p. 225–227°.

Anal.[3] Calcd. for $C_{12}H_8N_2O_2S$: C, 59.0; H, 3.3; N, 11.5. Found: C, 58.9; H, 3.2; N, 11.0.

2-Chloro-4-(3-coumarinyl)-thiazole, IV.—A mixture of 18 g. of III, 100 ml. of acetic acid, 200 ml. of concd. hydrochloric acid, and 40 ml. of butyl nitrite was made at 15° and allowed to stand at room temperature for twelve hours. The crystalline precipitate (10.5 g.) was then removed; an additional 1 g. was obtained by diluting the mother liquor. The product was distilled, b. p. 245–248° at 20 mm., and then recrystallized from acetic acid, giving fine white needles or coarse colorless prisms (9.5 g.), m. p. 170–171°.

Anal. Calcd. for $C_{12}H_6ClNO_2S$: C, 54.7; H, 2.3; N, 5.3. Found: C, 54.6; H, 2.3; N, 5.3.

When 1 g. of IV was warmed for ten minutes with 5 ml. of piperidine, and the mixture was then steam distilled, there was left a dark residue of **4-(3-coumarinyl)-2-(1-piperidyl)-thiazole.** The product, purified by distillation, b. p. 310–315° at 15 mm., and crystallization from alcohol, formed deep yellow prisms (0.9 g.), m. p. 132–133°.

Anal. Calcd. for $C_{17}H_{16}N_2O_2S$: N, 8.98. Found: N, 8.97.

Similar treatment of IV with aniline gave an anilino derivative (1.2 g.) which separated in a gelatinous form. Acetylation of the product by boiling it with acetic anhydride, and crystallization from acetic acid gave **2-(N-acetylanilino)-4-(3-coumarinyl)-thiazole acetate,** yellow prisms, m. p. 230–231°. The same substance was obtained by boiling 1 g. of III with 5 ml. of aniline and 0.1 g. of ammonium chloride for fifteen minutes, and then acetylating the product.

Anal. Calcd. for $C_{20}H_{14}N_2O_3S + C_2H_4O_2$: C, 60.2; H, 4.3. Found: C, 60.4; H, 4.1.

α-(2-Chloro-4-thiazolyl)-o-methoxycinnamic Acid, V.—When 4.7 g. of IV was boiled for five minutes with 2.5 g. of sodium hydroxide in 10 ml. of alcohol and 25 ml. of water, there was obtained a clear solution. This was cooled and treated with three 2-ml. portions of methyl sulfate and two 2-g. portions of sodium hydroxide, added alternately. Alcohol was removed by distillation; the mixture was acidified with hydrochloric acid, boiled for a few minutes, then basified with sodium carbonate. Unchanged IV (1.5 g.) was removed by filtration, and the filtrate was acidified. The precipitate (3.2 g.) was washed with ether, giving pale yellow crystals, m. p. 142–143°.

Anal. Calcd. for $C_{13}H_{10}ClNO_3S$: N, 4.7. Found: N, 4.9.

2-Chloro-4-thiazolecarboxylic Acid, VI.—A solution of 1.5 g. of V and 0.3 g. of sodium carbonate in 10 ml. of water at 20° decolorized rapidly about 70 ml. of 4% potassium permanganate. When the manganese dioxide was removed by filtration and the cloudy filtrate was kept at 0° for twelve hours, there separated about 200 mg. of crystalline *o*-methoxybenzaldehyde, identified by comparison with an authentic sample, and as its *p*-nitrophenylhydrazone.

The mother liquor was acidified with sulfuric acid and extracted with four 15-ml. portions of ether. There was obtained about 400 mg. of acidic product, colorless plates from water, m. p. 220–221° dec. Qualitative examination showed the presence of chlorine, nitrogen and sulfur.

Anal. Calcd. for $C_4H_2ClNO_2S$: C, 29.4; H, 1.2; N, 8.6. Found: C, 29.4; H, 1.4; N, 8.8.

3-(ω-Anilinoacetyl)-coumarin, VII.—A solution of 2.7 g. of II and 2 g. of aniline in 15 ml. of alcohol deposited 2.6 g. of red crystals when it was boiled for fifteen minutes. A pure product, red prisms, m. p. 180–185° with gas evolution, was obtained by recrystallization from toluene.

Anal. Calcd. for $C_{17}H_{13}NO_3$: N, 5.0. Found: N, 4.9.

The **acetyl derivative,** pale yellow plates from acetic acid, m. p. 181–182°, was obtained by boiling the anilino compound with acetic anhydride for five minutes.

Anal. Calcd. for $C_{19}H_{15}NO_4$: C, 71.0; H, 4.7. Found: C, 71.1; H, 4.7.

N-[β-(3-Coumarinyl)-β-oxoethyl]-pyridinium Bromide, VIII.—To a solution of 8 g. of II in 100 ml. of hot toluene was added 2.5 g. of pyridine. After the mixture had been kept for four hours at room temperature, it was filtered. The crystalline product (9.7 g.) formed pale yellow plates from acetic acid, dec. *ca.* 218°.

Anal. Calcd. for $C_{16}H_{12}BrNO_3$: C, 55.5; H, 3.5. Found: C, 55.8; H, 3.8.

When a solution of the bromide in hot water was treated with excess sodium hydroxide, a brown gelatinous precipitate was formed that did not change when the mixture was boiled. When dried, this material formed scales similar in appearance to ferric hydroxide. It was soluble in hydrobromic acid with regeneration of the bromide.

N-[β-(3-Coumarinyl)-β-oxoethyl]-2-methylpyridinium bromide formed yellow-brown prisms from alcohol, dec. *ca.* 200°.

(2) *Cf.* Babcock and Fuson, THIS JOURNAL, **55**, 2946 (1933).

(3) The author thanks Mr. Roger Amidon and Mr. Wm. Cummings for most of the analyses in this paper.

Anal. Calcd. for $C_{17}H_{14}BrNO_3$: C, 56.7; H, 3.9. Found: C, 57.0; H, 4.2.

N-[β-(3-Coumarinyl)-β-oxoethyl]-quinolinium bromide formed orange-brown prisms from water or from acetic acid–ethyl acetate, dec. *ca.* 210°.

Anal. Calcd. for $C_{20}H_{14}BrNO_3$: C, 60.6; H, 3.6. Found: C, 61.0; H, 3.9.

3-Carbethoxy-N-[β-3-(coumarinyl)-β-oxoethyl]-pyridinium bromide formed nearly colorless plates from water containing a little hydrobromic acid, dec. *ca.* 190°.

Anal. Calcd. for $C_{19}H_{16}BrNO_5$: C, 54.5; H, 3.8. Found: C, 55.1; H, 4.3.

4-Carbethoxy-4-[β-3-(coumarinyl)-β-oxoethyl]-pyridinium bromide formed fine nearly colorless needles from alcohol, dec. *ca.* 170°.

Anal. Calcd. for $C_{19}H_{16}BrNO_5$: C, 54.5; H, 3.8. Found: C, 54.3; H, 4.2.

Summary

The substance obtained by the action of bromine on 3-acetylcoumarin and described previously as 3-acetyl-4-bromocoumarin is actually 3-bromoacetylcoumarin, for it yields a substituted thiazole when it is treated with thiourea. It reacts with aniline to form an anilino derivative whose red color is anomalous, and with pyridine to form an addition compound which is not cleaved by aqueous alkali.

MINNEAPOLIS, MINNESOTA RECEIVED DECEMBER 12, 1949

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Studies in Enzyme Inhibition. I. Action of Some Simple Pterines on Xanthine Oxidase

BY H. G. PETERING AND J. A. SCHMITT

Wieland and Liebig[1] showed that xanthine oxidase catalyzes the oxidation of xanthopterine to leucopterine about half as rapidly as it does hypoxanthine or xanthine. Recently Kalckar and Klenow[2] and Kalckar, Kjeldgaard and Klenow[3] found that 2-amino-4-hydroxy-6-formylpterine (I) inhibits the enzymatic oxidation of hypoxanthine, xanthine and xanthopterine. The activity reported by Kalckar, *et al.*,[2,3] for I is of such very high order as to suggest great specificity.

We have investigated the relationship of structure to the inhibitory action of a number of simple pterines on the xanthine oxidase system of milk and rat liver, and wish to report the results of one phase of our work which indicates the highly specific chemical nature of this inhibition.

Experimental

Materials.—The xanthine oxidase preparations were either a crude milk enzyme made according to the method of Dixon and Kodoma[4] or a rat liver preparation obtained by homogenization in a Waring Blendor.

Hypoxanthine (Schwarz Laboratories) and xanthine (Eastman Kodak Co.) were used without further purification. The pterines and 2,6-diaminopurine were synthesized by methods which will be published elsewhere.

Procedure.—All studies were made with the Warburg respiration apparatus at 38°. With the milk enzyme the method of Ball[5] was closely followed. With rat liver homogenates a procedure similar to that of Potter and Elvehjem[6] was used. The inhibitor was dissolved in 0.15 ml. of 0.01 *N* sodium hydroxide and either added directly to the enzyme at the beginning of the experiment or mixed with the xanthine solution in the side arm.

Results

Preliminary experiments with the milk enzyme confirmed the reports of Wieland and Liebig[1] and Kalckar, *et al.*,[2,3] in that the enzyme preparation which was active with hypoxanthine and xanthine activated the oxidation of xanthopterine and its activity was markedly inhibited by I. In addition it was found that the enzyme would activate the oxidation of 2-amino-4-hydroxypterine at a rate about twice the rate of the oxidation of xanthopterine.

A more detailed study with a number of different pterines revealed important differences in the effect of these compounds on the enzymatic oxidation of hypoxanthine. These data are given in Table I. The strikingly effective inhibition of hypoxanthine oxidation by 2-amino-4-hydroxy-6-hydroxymethylpterine (II), which is similar in its activity to I is readily seen. The data with milk xanthine oxidase shown in Table I are insufficient to make a direct comparison of the activities of I and II with the less active compounds; such a comparison would require information on the minimum inhibitor concentration for optimum activity against this type of enzyme preparation. However, the data of Table I are qualitatively consistent with other data on liver homogenates presented below, wherein such a comparison was possible.

Having confirmed the effect of I on xanthine oxidase of milk and noted differences between several types of pterines, this inhibition was studied more extensively with the rat liver enzyme. In this work a larger series of compounds was used and these compounds were studied over a wider range of inhibitor concentration. Data for these experiments are given in Table II and Figs. 1–3.

The data in Table II may be more readily evaluated and a number, which is reasonably indicative of the relative inhibition index of the compound, may be obtained if the inhibition curve for I shown in Fig. 1 is used as a reference and the

(1) Wieland and Liebig, *Ann. Chem.*, **555**, 146 (1944).
(2) Kalckar and Klenow, *J. Biol. Chem.*, **172**, 349 (1948).
(3) Kalckar, Kjeldgaard and Klenow, *ibid.*, **174**, 771 (1948).
(4) Dixon and Kodoma, *Biochem. J.*, **20**, 1104 (1926).
(5) Ball, *J. Biol. Chem.*, **128**, 5 (1939).
(6) Potter and Elvehjem, *ibid.*, **114**, 495 (1936).