Anal.—Calcd. for $C_{22}H_{42}N_4S_4$: C, 53.81; H, 8.62; N, 11.41. S, 26.13. Found: C, 53.90; H, 8.45; N, 11.16; S, 26.35.

1-Methyl-3-phenethylthiourea—To a cooled solution of 1.21 g. (0.01 mole) of β-phenethylamine in 5 ml. of ethanol was added 0.73 g. (0.01 mole) of methyl isothiocyanate. After an evolution of heat, water was added dropwise to a lasting turbidity. The mixture was stored in the refrigerator overnight, and the precipitate was filtered, washed with ethanol, and air-dried, giving 69% of white product; m.p. 61–63°. IR(Nujol)975(C=S), 1300(C=S)cm.$^{-1}$.

Anal.—Calcd. for $C_{10}H_{14}N_2S$: C, 61.80; H, 7.26; N, 14.42. Found: C, 62.18; H, 7.19; N, 14.15.

1-Methyl-3-(4-nitrophenethyl)-thiourea—To a cooled solution of 1.66 g. (0.01 mole) of 4-nitro-β-phenethylamine (9) in 5 ml. of ethanol was added slowly with stirring 0.73 g. (0.01 mole) of methyl isothiocyanate in 2 ml. of ethanol. The solution was refluxed for one hr., cooled, and stored in the refrigerator overnight. The white solid was collected, washed with ethanol, and air-dried; giving a 75% yield m.p. 79–82°.

Anal.—Calcd. for $C_{10}H_{13}N_3O_2S$: C, 50.18; H, 5.48; N, 17.56. Found: C, 50.42; H, 5.48; N, 17.30.

1-Methyl-3-(4-methylsulfonamidoethylphenyl)-thiourea—A mixture of 0.530 g. (0.0025 mole) of 4-methylsulfonamidoethylaniline and 0.185 g. (0.0025 mole) of methyl isothiocyanate was heated on a steam bath until it became liquid. Absolute ethanol (10 ml.) was added, and the mixture was refluxed until it became homogeneous. After being cooled, the crystalline solid was collected, washed with ethanol, and dried *in vacuo*, giving an 83% yield; m.p. 179–181°.

Anal.—Calcd. for $C_{11}H_{17}N_3O_2S_2$: C, 45.95; H, 5.96; N, 14.62. Found: C, 46.04; H, 5.92, N, 14.27.

1-Methyl-3-(4-trifluoroacetamidoethylphenyl)-thiourea—A mixture of 1.16 g. (0.005 mole) of 4-trifluoroacetamidoethylaniline and 0.365 g. (0.005 mole) of methyl isothiocyanate in 10 ml. of ethanol was refluxed for 1 hr. The solution was cooled, and the white solid was collected, washed with ethanol, and dried *in vacuo*, giving an 86% yield; m.p. 167–170°.

Anal.—Calcd. for $C_{12}H_{14}F_3N_3OS$: C, 47.21; H, 4.62; N, 13.76. Found: C, 47.07; H, 4.90; N, 13.80.

N,N′-Bis(N-methylthiocarbamido)-β-4-aminophenethylamine—Methyl isothiocyanate (1.46 g., 0.02 mole) was added slowly to β-4-aminophenethylamine (1.36 g., 0.01 mole) (Aldrich Chemical Co.) with ice-cooling. After an evolution of heat, ethanol (25 ml.) was added, and the mixture was refluxed until a solution resulted. After being cooled, a white solid appeared which was collected, recrystallized from aqueous ethanol, and air-dried, giving an 80% yield; m.p. 185–190°.

Anal.—Calcd. for $C_{12}H_{18}N_4S_2$: C, 51.03; H, 6.42; N, 19.84. Found: C, 51.13; H, 6.55; N, 19.45.

REFERENCES

(1) M. Goldstein, B. Anagnosti, E. Lauber, and M. McKereghan, *Life Sci.*, **3**, 763(1964); J. Musacchio, I. J. Kopin, and I. Snyder, *ibid.*, **3**, 769(1964).

(2) A. Carlsson, M. Lindquist, K. Fuxe, and T. Hokfelt, *J. Pharm. Pharmacol.*, **18**, 60(1966).

(3) J. Jonsson, H. Grobecker, and L.-M. Gunne, *ibid.*, **19**, 201 (1967).

(4) A. K. Pfeifer, E. Galambos, and L. Gyorgy, *ibid.*, **18**, 254 (1966).

(5) K. D. Krantz and L. S. Seiden, *ibid.*, **20**, 166(1968).

(6) A L. Green, *Biochim. Biophys. Acta*, **81**, 394(1964).

(7) J. S Buck and C S. Leonard, *J. Am. Chem. Soc.*, **53**, 2688 (1931).

(8) A. E. S. Fairfull and D. A. Peak, *J. Chem. Soc.*, **1955**, 796.

(9) P. Ehrlich and P. Pistschimuka, *Ber.*, **45**, 2428(1912).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 4, 1969 from the *Department of Chemistry, Massachusetts College of Pharmacy, Boston, MA 02115*

Accepted for publication August 12, 1969.

Presented to the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

Abstracted from a thesis submitted by J. C. Anderson to the Massachusetts College of Pharmacy in partial fulfillment of Ph.D. degree requirements.

The authors express their appreciation to Smith Kline & French Laboratories for financial support.

Biosynthesis of Rubrofusarin by *Fusarium graminearum*

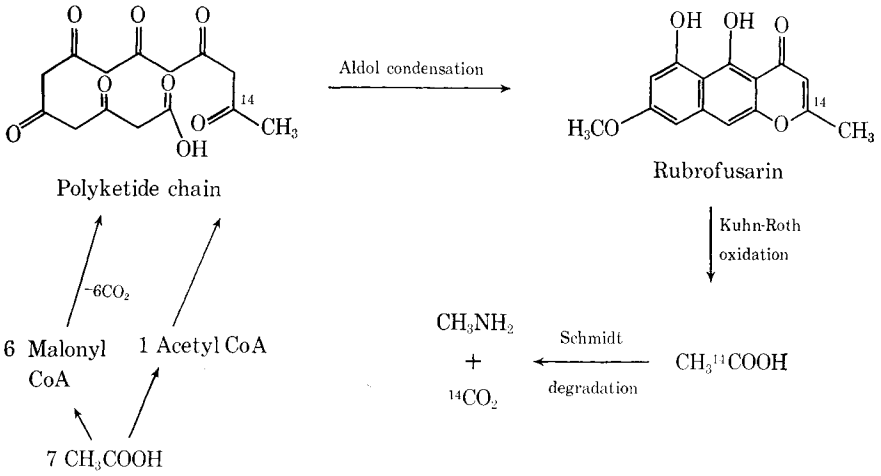
BRUCE H. MOCK* and JAMES E. ROBBERS

Abstract □ Using radioactively labeled acetate, evidence was obtained to support the hypothesis that the biosynthesis of rubrofusarin proceeds through a polyketide chain intermediate. It was shown that labeled acetate when diluted with nonlabeled malonate, is preferentially incorporated into the terminal acetate starting unit of the polyketide chain.

Keyphrases □ *Fusarium graminearum*—rubrofusarin biosynthesis □ Acetate-malonate condensation—rubrofusarin formation □ Rubrofusarin, biosynthesis—polyketide chain intermediate

Rubrofusarin is an orange-red pigment produced by the fungus, *Fusarium graminearum* Schwabe. This organism is the imperfect stage of *Gibberella zeae* (Schweinitz) Petch, a common plant pathogen. Rubrofusarin was first thought to be a xanthone derivative, as reported by Ashley *et al.* (1), but was later discovered by Tanaka and Tamura (2) to be a derivative of 2-methylnaphtho-γ-pyrone.

Several hypotheses can be proposed for the biosynthesis of rubrofusarin. One theory is that it is a product of a biosynthetic pathway involving shikimic acid and mevalonic acid, as are some of the anthraquinones found in higher plants (3). The most likely proposal, however, is that rubrofusarin is formed by an acetate-malonate condensation with the formation of a polyketide chain which cyclizes to give the tricyclic ring system (Scheme I). Shibata and Ikekawa (4) have shown that rugulosin, a fungal anthraquinone dimer, was biosynthetically formed by the head-to-tail linkage of 14 malonate units and two units of acetate with release of carbon dioxide from each malonate. In each of the tricyclic monomers one of the acetate units served as a terminal starting unit in building the polyketide chain.



Scheme I

The purpose of this work was to investigate whether the acetate-malonate hypothesis could be used in explaining the biosynthesis of rubrofusarin.

EXPERIMENTAL

Fusarium graminearum was transferred from a Czapek-Dox slant culture to 100 ml. glucose-mannitol-brewer's yeast medium (2, 2, and 1%) and grown in shake culture for 1 week at 27° to serve as the inoculum. The fungus used in the experiment was obtained by pipeting 2 ml. of inoculum into 100-ml. shake cultures of NL-406 culture medium (5). After 6 days of growth at 27°, 2.4 μc. of sodium acetate, 1-^{14}C (0.12 μmoles) was aseptically added to each of 20 cultures. Simultaneously, 0.1 mmoles of diethyl malonate (1.0 ml. from a 1.6% w/v solution in 5% ethanol) was aseptically added to 11 of the cultures. In harvesting the cultures 72 hr. later, they were divided into two groups, Lot *A* contained 9 acetate cultures and 10 carrier cultures (cultures grown under the same conditions for production of nonlabeled carrier rubrofusarin), and Lot *B* contained 11 acetate plus malonate cultures and 7 carrier cultures. The filtered mycelia were oven-dried at 60° and ground to a powder in a large mortar and pestle.

Rubrofusarin from each lot was obtained by extracting the powdered mycelia with petroleum ether (b.p. 30–60°) in a continuous extraction apparatus for 7 days. Evaporating the extract to near dryness resulted in the appearance of crystals overnight. These were redissolved in hot benzene and the orange-red crystals which appeared on standing were filtered, washed with cold petroleum ether, and air dried. Identification of rubrofusarin was confirmed by melting point (1) and by its mass spectrum.

Approximately 1 mg. of rubrofusarin from each of the two lots of crystals was dissolved in 10 ml. of toluene counting solution for counting in a liquid scintillation counter. In each case the specific activity of rubrofusarin was determined.

The remainder of each group of rubrofusarin crystals including a measured amount of carrier material was degraded by the Kuhn-Roth oxidation procedure (6). Acetic acid liberated by the reaction was removed by distillation in a Wiesenberger apparatus and determined by titration with 0.02 *N* sodium hydroxide. The sodium acetate solution thus obtained was evaporated to dryness and transferred to a counting vial by washing with water, then with Bray's counting solution. Specific activity of the acetate was determined in a liquid scintillation counter.

An aliquot containing 115 μmoles of acetate obtained from Kuhn-Roth oxidation of Lot *A* rubrofusarin was subjected to a Schmidt degradation (7) to determine if the radioactive label remained in the carboxyl carbon of acetate.

DISCUSSION

The results of this study show that acetate was definitely incorporated. See Table I. The percent of incorporation was low due to the fact that acetate was probably utilized by countless other metabolic pathways.

Seven acetate units should be required to form the carbon skeleton of rubrofusarin. One acetate unit, first being converted to acetyl-CoA, should serve as the terminal starting unit; six acetate units should be converted to malonyl-CoA which should then be added to the initial acetyl-CoA in a "head-to-tail" fashion with concomitant release of carbon dioxide (Scheme I). Theoretically, 14.3% (1/7) of the total radioactivity of rubrofusarin should be in the starting unit acetate. This was the acetate obtained by the Kuhn-Roth oxidation. The result of 20.4% of total activity was reasonable when one assumes that conversion of acetate to malonyl-CoA required one more reaction than the formation of acetyl-CoA, resulting in a possible metabolic dilution of acetate. Additionally, labeled malonyl-CoA might be diluted with nonlabeled malonyl-CoA. Thus, because of endogenous metabolism, a greater quantity of radioactive acetate should appear in the starting unit than in the malonyl-CoA derived portion of the molecule.

In order to add evidence to the possibility of the above explanation, a series of cultures was flooded with nonlabeled malonate (in the form of diethyl malonate) simultaneously with the addition of

Table I—Experimental Results

	Acetate Only	Acetate + Nonlabeled Malonate
Activity fed/culture	5.23×10^6 dpm	5.23×10^6 dpm[a]
Incorporation, %	0.26	0.35
Rubrofusarin	1.29×10^6 dpm/m*M*	1.23×10^6 dpm/m*M*
Kuhn-Roth acetate activity	2.64×10^5 dpm/m*M*	5.84×10^5 dpm/m*M*
Kuhn-Roth acetate % of total rubrofusarin activity	20.4	47.5
Theoretical, %	14.3 (1/7)	100.0
Schmidt degradation % activity of acetate in carboxyl carbon atom	98.6	

[a] Disintegrations per minute.

radioactive acetate. This procedure should cause the dilution of radioactive malonyl-CoA formed from radioactive acetate. (Also, the added malonate might alter the acetate-malonate equilibrium to prevent incorporation of radioactive label into malonyl-CoA.) If no labeled acetate were converted to malonyl-CoA, 100% of the rubrofusarin molecule's radioactivity would be in the acetate of the terminal starting unit.

Flooding the cultures with nonradioactive malonate increased the amount of radioactivity of the carbon in Position 2 of the ring system of rubrofusarin; this supports the view that acetate serves as the terminal starting unit in the formation of a polyketide chain. Since the increase in percent incorporation was 47.5% instead of the theoretical 100%, apparently, some radioactive acetate was converted to malonyl-CoA, decreasing the percentage value of the experimental results. Addition of nonlabeled malonate, however, caused a significant increase in radioactivity of carbon in Position 2 of rubrofusarin by 133% over the results of the acetate only experiment.

Schmidt degradation of acetate recovered from the Kuhn-Roth oxidation of radioactive rubrofusarin revealed no randomization of radioactive label in the terminal starting acetate unit. Absence of such randomization indicated that the original radioactive acetate had not been metabolized to another compound before incorporation into rubrofusarin

REFERENCES

(1) J. N. Ashley, B. C. Hobbs, and H. Raistrick, *Biochem. J.*, **31,** 386(1937).

(2) H. Tanaka and T. Tamura, *Tetrahedron Letters*, **1961,** 151.

(3) M. H. Zenk and E. Leistner, *Lloydia*, **31,** 275(1968).

(4) S. Shibata and T. Ikekawa, *Chem. Ind. London*, **1962,** 360.

(5) H. G. Floss and D. Gröger, *Z. Naturforsch.*, **18b,** 519(1963).

(6) H. Simon and H. G. Floss, "Anwendung von Isotopen in der Organischen Chemie and Biochemie," vol. 1, Springer-Verlag, Berlin, West Germany, 1967, p. 13.

(7) *Ibid.*, p. 26.

ACKNOWLEDGMENTS AND ADDRESSES

Received June 4, 1969 from the *Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication July 31, 1969.

The authors thank the National Science Foundation for financial support, Dr. Heinz Floss and Dr. Egil Ramstad for valuable discussions and encouragement, and Dr. Helmut Guenther for performing the Schmidt degradation.

* Winner of the 1969 Kilmer Prize and National Science Foundation Undergraduate Research Participant, 1967.

Effect of Salts on the Surface/Interfacial Tension and Critical Micelle Concentration of Surfactants

LUCY S. C. WAN and PHILIP K. C. POON

Abstract □ All the salts used produced shifts of the CMC to lower concentrations and reduced the surface/interfacial tensions of air-surfactant solution and liquid paraffin-surfactant solution systems, respectively. No appreciable difference was observed when air was substituted for liquid paraffin as the upper phase, indicating that the hydrocarbon layer exerted no pressure effects. It appeared that shifts of CMC were related to the valency of the gegenion, a divalent gegenion would produce a shift much greater than a monovalent gegenion. Cationic gegenions were more effective in lowering the CMC of sodium lauryl sulfate than similar anions while anionic gegenions were effective with cetrimide and cetylpyridinium chloride. The CMC of cetomacrogol 1000 was practically unaffected by the addition of salt and the extent of interfacial tension reduction with respect to salt concentration was small when compared to corresponding systems containing ionic surfactants.

Keyphrases □ Surface-interfacial tension—salts effect □ Critical micelle concentration, surfactants—salts effect □ Interfacial, surface tension determinations—surfactant, salt-surfactant solutions

Anionic surfactants have been shown to be influenced by the type, magnitude of charge, and degree of hydration of the gegenions (1, 2), whereas cationic surfactants appear to be influenced only by the ionic size or degree of hydration of the gegenion (1, 3, 4). Nonionic surfactants although not charged may form hydroxonium ions with weak cationic properties (5) and their CMC's are affected by anions and their degree of hydration (5–10). It has been found that electrolytes promote adsorption and aggregation processes, possibly by decreasing the electrokinetic repulsion (5, 11–15) which can also be due to the screening of the double layer. Salts have been shown to suppress the dissociation of the surfactant monomer and cause a decrease in the desorption rate (16, 17). Greshfeld (18) attributes the decrease in surface tension to the formation of a monolayer of nondissociated monomers. Tartar (16, 19) postulates that addition of electrolyte would reduce the thickness of the ionic atmosphere enveloping the surfactant monomer.

Nonionic surfactants are characterized by their undissociation and higher degree of hydration. The latter property is indicated by an increase in the CMC with increase in the hydrophobic chain length (20, 21) and by the large positive values of the heat and entropy of micellization in the presence of salts as compared with ionic surfactants in the same concentration of salt (22). This communication reports the change in CMC and the further reduction of surface/interfacial tension of an air-liquid system and a liquid-liquid system where one phase is a surfactant solution, in the presence of added salts. The information obtained may be useful for the preparation of solubilized and emulsified products.

EXPERIMENTAL

Materials—The following salts were used: ammonium chloride BP, ammonium bromide BPC, ammonium sulfate,[1] lithium chloride,[1] lithium sulfate,[2] magnesium chloride,[3] magnesium sulfate

[1] E. Merck, Darmstadt, Germany.
[2] A. R. Grade, British Drug House Ltd.
[3] May and Baker, Dagenham, England.