[CONTRIBUTION FROM THE WYETH INSTITUTE FOR MEDICAL RESEARCH]

An Improved Synthesis of Mercaptomerin[1]

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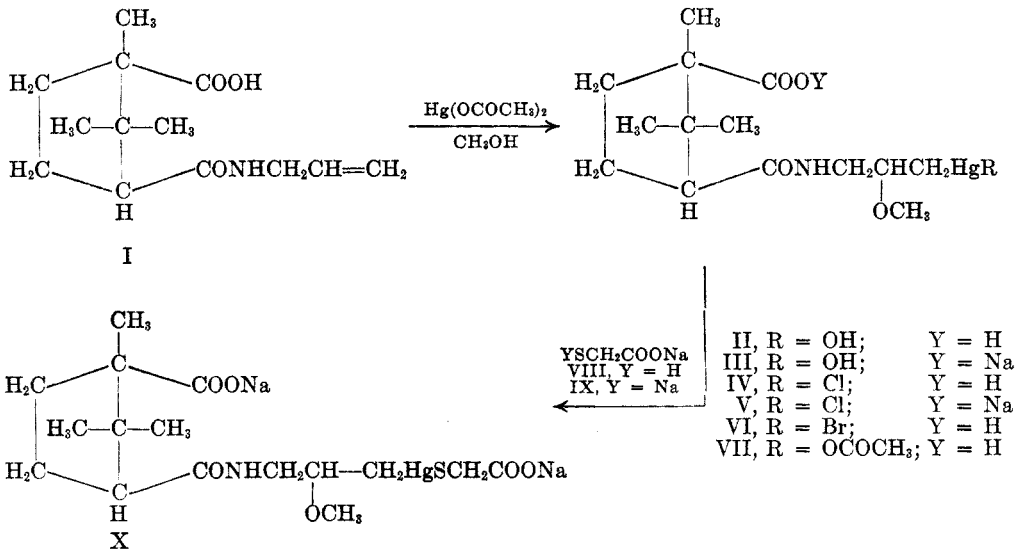
N-(3-Chloromercuri-2-methoxypropyl)-dl,α-camphoramic acid (IV) has been synthesized and found to be a useful intermediate for the synthesis of mercaptomerin (X). By reacting the sodium salt of IV with disodium thioglycolate in anhydrous methanol, X was formed in good yield and was isolated by the addition of acetone. Fractional crystallization from methanol-acetone gave X in crystalline form and in high purity. Aqueous solutions of X were found to have maximal stability at *p*H 9.5 containing 0.01% of EDTA.

Mercaptomerin (X) has been shown clinically to be an effective parenteral diuretic. The synthesis of X is shown by the following sequence of reactions.



Lehman[2] prepared X in aqueous solution by reacting sodium *N*-(3-hydroxymercuri-2-methoxypropyl)-α-camphoramate (III)[3] with one equivalent of sodium thioglycolate (VIII). Removal of the water by freeze-drying gave X as an amorphous product which was not further purified. Mercaptomerin, prepared by this procedure, is relatively stable in solid form; however, this material deteriorates rapidly in aqueous solution. Replacement of R in III by the thioglycolic acid sodium (VIII) group produced a mercurial diuretic (X) with low cardiac toxicity but of high sensitivity to traces of impurities, particularly heavy metals.

Our own experiments with this procedure revealed that it has distinct limitations. We tried a number of alterations but none of these involving II gave a satisfactory product. One disadvantage of the process using II as an intermediate is that II is a viscous material that shows no tendency to crystallize. Therefore, purification of the material was extremely difficult.

On the basis of this and other observations it was decided to investigate the possibility of developing analogs of II that could be easily isolated and purified. Two mercurials with these desired properties were found in the halomercuri compounds IV and VI. They were prepared by methoxymercuration of *N*-allyl-dl,α-camphoramic acid (I). The acetoxymercuri compound (VII) was not isolated but was directly treated with sodium chloride yielding *N*-(3-chloromercuri-2-methoxypropyl)-dl,α-camphoramic acid (IV). From reaction of VII with sodium bromide the corresponding bromo compound VI was isolated. Both compounds were obtained in pure crystalline form.

The following steps were carried out in anhydrous methanol and in an atmosphere of nitrogen to avoid metal-catalyzed oxidation of thioglycolic acid[4] to dithiodiglycolic acid since the latter has been found to decrease considerably the stability of X.[5]

(1) Trade name: Thiomerin® (Wyeth).

(2) R. A. Lehman, *Proc. Soc. Exptl. Biol. Med.*, **64**, 428 (1947); U. S. Patents **2,576,349** (1951) and **2,675,388** (1954); Brit. Patent **732,433** (1955).

(3) N. M. Molnar, U. S. Patent **2,117,901**.

(4) R. Andreasch, *Ber.*, **12**, 1390 (1879); H. Wieland and W. Franke, *Ann.*, **464**, 155 (1928); A. E. Martell and M. Calvin, *Chemistry of the Metal Chelate Compounds*, Prentice-Hall, Inc., New York, N. Y., 1952, p. 384.

(5) G. Wendt, U. S. Patent **2,751,325**, (1956).

The chloromercuri compound IV was converted to the sodium salt V by reaction with sodium methoxide in methanol. V gave X upon treatment with disodium thioglycolate (IX). Since the reaction was accompanied by the evolution of heat the reaction mixture was cooled in an ice bath to maintain the temperature between 15–25° and thereby to minimize side reactions. X was precipitated by the addition of acetone, filtered off, and dried in vacuo. Fractional crystallization from methanol-acetone yielded very pure X[6a] in fine needles. Evidence of the purity of the crystalline X was provided by stability data.

For tracer studies[6b] with X using Hg^{203} only slight modifications of the described procedure were necessary.

Stability studies. The stability of X in aqueous solution depends not only on the purity of the material but also on the *p*H. Maximal stability was found in the *p*H range 9.0–9.5.[7] Lowering of the *p*H reduced the stability, *e.g.* *p*H 8.0 decreased the stability by 2 days. At a *p*H below 7.0 the deterioration was considerably accelerated. All the stability studies were conducted at 50°. The results of the data obtained at *p*H 9.0–9.5 are presented in Table I. Comparative stability studies with amorphous X and crystalline X revealed that the former was about half as stable as the crystalline X. The stability of the latter could be considerably increased by the addition of small amounts of ethylenediaminetetraacetic acid (EDTA).[8] Addition of nitrilotriacetic acid (NTA) proved less suitable than EDTA.

TABLE I

X	Days	Chelating Agents
Lehman method	$4^1/_2$–$5^1/_2$	
Crystalline	11–12	
Crystalline	19–29	0.01% EDTA
Crystalline	19–24	0.001% EDTA
Crystalline	15–16	0.1% NTA

The two complexing agents EDTA and NTA are known to form relatively stable chelates with metals, *e.g.* Cu, Ca, and Mg. Spectrochemical analysis of crystalline X showed the presence of traces of these elements.

(6a) G. Wendt, U. S. Patent **2,834,795** (1958).

(6b) J. K. Aikawa, A. J. Blumberg, and D. A. Catterson, *Proc. Soc. Exptl. Biol. and Med.*, **89**, 204 (1955); J. P. DeMetry and J. K. Aikawa, *Proc. Soc. Exptl. Biol. and Med.*, **90**, 413 (1955); J. K. Aikawa and W. R. Carlson, *Am. J. Med. Sci.*, **230**, 622 (1955); J. K. Aikawa, *Clin. Research Proceedings*, **3**, 55 (1955); J. K. Aikawa and R. H. Fitz, *J. Clin. Invest.*, **35**, 775 (1956); J. K. Aikawa, *Am. J. Med. Sci.*, **235**, 179 (1958).

(7) Stability at a *p*H higher than 9.5 was not studied since solutions at this *p*H range would generally cause skin irritations at the site of injection.

(8) P. Pfeiffer and W. Offerman, *Ber.*, **75**, 1 (1942). P. Pfeiffer and H. Simons, *Ber.*, **76**, 847 (1943). G. Schwarzenbach, *Helv. Chim. Acta*, **35**, 2344 (1952).

EXPERIMENTAL

All melting points and boiling points are uncorrected.

Equipment. Since even traces of heavy metals influence the purity and the stability of mercaptomerin all the equipment used in the process was made of glass or porcelain. Glassware should be cleaned carefully, preferably with a hot mixture of sulfuric and nitric acids. It was rinsed with double distilled (all glass apparatus) water and dried.

Chemicals. Nitrogen (Seaford Grade, oxygen free, from Air Reduction Sales Corp.). Thioglycolic acid (The Matheson Co.) was freshly distilled in vacuo (b.p. 84–85°, 2 mm.) under nitrogen and kept under nitrogen. Methanol, acetone, mercuric oxide, and mercuric acetate were analytical reagent Mallinckrodt. Sodium methylate was from the Mathieson Alkali Works.

N-Allyl-dl,α-camphoramic acid (I) was prepared by the procedure of Wootton[9] for *N*-allyl-*d*,α-camphoramic acid. After recrystallization from aqueous methanol I melted at 174–175° (d-form melts at 157–158°).

Anal. Calcd. for $C_{13}H_{21}NO_3$: C, 65.23; H, 8.84; N, 5.85. Found: C, 65.61; H, 9.11; N, 5.77.

N-(3-chloromercuri-2-methoxypropyl)-dl,α-camphoramic acid (IV). A suspension of 95.7 g. (0.30 mole) of mercuric acetate in 75 ml. of methanol was stirred for 0.5 hr. in a 3-necked flask equipped with stirrer, dropping funnel, drying tube, and thermometer. To this suspension was added dropwise and with stirring, a solution of 71.7 g. (0.30 mole) of I in 200 ml. of methanol over a period of 30 min. The temperature of the reaction mixture was kept below 30°. Stirring was continued for 1 hr. and thereafter the solution was refluxed for 4 hr. on a steam bath. After the reaction mixture had attained room temperature a solution of 17.7 g. (0.30 mole) of sodium chloride in 75 ml. of water was added. Stirring was continued for 1 hr. at 25° and the solution was then refluxed for 1 hr. The small amount of grey precipitate was removed by centrifuging. The clear supernatant was concentrated on a steam bath to about half of its original volume and then added dropwise to 900 ml. of ice water with vigorous stirring and the stirring was continued until the precipitate became granular. After filtering with suction the material was washed with 300 ml. of cold water and dried over a mixture of anhydrous calcium chloride and potassium hydroxide pellets yielding 135 g. (89%) of IV. After 2 recrystallizations from acetone-water the product melted at 133–134° (dec.).

Anal. Calcd. for $C_{14}H_{24}ClHgNO_4$: Hg, 39.61; OCH_3, 6.12; Found: Hg, 39.86; OCH_3, 6.09.

N-(3-Bromomercuri-2-methoxypropyl)-dl,α-camphoramic acid (VI). To a mixture of 21.7 g. (0.10 mole) of mercuric oxide and 6.7 ml. of glacial acetic acid was added a solution of 23.9 g. (0.10 mole) of I in 100 ml. of methanol. The reaction mixture was stirred for 0.5 hr. and was then refluxed for 1 hr. Some insoluble material was removed by filtration and a solution of 10.3 g. (0.10 mole) of sodium bromide in 25 ml. of water was added to the warm filtrate. After clarifying by filtration the solution was added dropwise and with stirring to 600 ml. of water. The granular precipitate after washing with water and drying over anhydrous calcium chloride amounted to 72%. After two recrystallizations from acetone-water the material melted at 124–125°.

Anal. Calcd. for $C_{14}H_{24}BrHgNO_4$: Hg, 36.42; Br, 14.51; N, 2.54. Found: Hg, 36.52; Br, 13.96; N, 2.52.

Mercaptomerin (X). Disodium salt of *N*-(3-carboxymethylthiomercuri-2-methoxypropyl)-dl,α-camphoramic acid. (*a*) *Solution of* V. Fourteen g. (0.26 mole) of sodium methoxide was added with stirring in small portions to 250 ml. of anhydrous methanol. To the resulting turbid solution was added 101.3 g. (0.20 mole) of crude pulverized IV in small portions with stirring over a period of 30 min. The temperature of the reaction mixture should not rise above

(9) W. O. Wootton, *J. Chem. Soc.*, **97**, 408 (1910).

30°. A small amount of insoluble material was removed by centrifugation. The slightly yellow but clear supernatant was decanted and the centrifuge bottle was rinsed with 20 ml. of methanol. The *p*H of the combined supernatants was 9.0–9.5.[10] If the solution has a *p*H lower than 8.5, it should be properly adjusted by the addition of solid sodium methoxide in small portions.

(*b*) *Solution of* IX. All the following steps were conducted under nitrogen. To 18.4 g. (0.20 mole) of freshly distilled thioglycolic acid in 200 ml. of anhydrous methanol was added 23.2 g. (0.43 mole) of sodium methoxide in small portions with stirring. The temperature was kept between 15–20°. The resulting solution was opalescent and slightly pink. It was poured into a separatory funnel which had been deaerated with nitrogen. The flask was rinsed with 30 ml. of methanol. The *p*H of the combined methanol solution was 11.

The solution of V was poured into a 3-necked flask provided with a calcium chloride tube. After flushing the apparatus with nitrogen the solution of IX was added dropwise with vigorous stirring over a period of 1 hr. The temperature of the reaction mixture was kept between 15 and 20° by applying an ice water bath. The reaction mixture was then stirred for 3 hr. and was allowed to stand for 20 hr. at room temperature. It was then centrifuged and the bottle was rinsed with 20 ml. of methanol. The combined clear supernatants gave a negative nitroprusside test and the *p*H was between 10 and 11. The solution was added dropwise to 2.5 l. of pure acetone over a period of 1 hr. The white precipitate was collected on a Buchner funnel. The flask was rinsed twice with a mixture of 50 ml. of methanol and 250 ml. of acetone. The white cake was finally washed with 500 ml. of acetone and sucked dry. It was quickly transferred to a desiccator and the adhering solvents were removed at 25° by applying an oil pump vacuum (0.1 mm.) overnight. The yield of crude X was approximately 87 g. (70–80%). By using pure, recrystallized IV the yield of X could be increased to 86% of a more stable product.

(10) Measured by adding one drop of this solution to pHydrion papers moistened with water.

Recrystallization of X. The 86.6 g. of crude, pulverized X was dissolved in 433 ml. of methanol by gentle heating (50°) on a water bath with stirring. A clear colorless solution resulted. To the warm methanol solution acetone (approximately 1000 ml.) was added in small portions with stirring until the mixture became turbid. The white precipitate was filtered off quickly by gravity and discarded. To the filtrate more acetone was added with stirring until a permanent turbidity occurred and the first crystals were formed. The stoppered Erlenmeyer was allowed to stand at room temperature in the dark for 20 hr. The crystalline precipitate of X was filtered, washed with 600 ml. of acetone, and dried in vacuo (oil pump) overnight, and finally over phosphorus pentoxide at 0.1 mm. yielding approximately 62 g. The compound is extremely hygroscopic. For analysis a sample was dried over phosphorus pentoxide at 0.1 mm. and 80° for 3 hr.

Anal. Calcd. for $C_{16}H_{25}HgNNa_2O_6S$: Hg, 33.1; S, 5.3. Found: Hg, 33.3; S, 5.3.

Stability studies at 50 ± 3°. The test solutions were prepared by dissolving 242 mg. of X in 2 ml. of distilled water. The mercury content of these solutions corresponds to that of the solutions prepared for injection. The *p*H of the solutions was, if necessary, adjusted to 9.0–9.5 with 0.1*N* sodium hydroxide.

In experiments with EDTA the calculated amount of the disodium salt of EDTA (disodium versenate, analytical reagent, Bersworth) was dissolved in water. The *p*H was adjusted to 8 with dilute sodium hydroxide before X was added. The *p*H after the addition of X was 9.0–9.5.

Most of the solutions decomposed by forming a black precipitate of HgS. The supernatant remained clear. In some solutions evidence of deterioration consisted of a yellow precipitate which after some time turned black. The supernatant of the latter samples was turbid.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF MEDICINE, YALE UNIVERSITY]

Synthesis and Physical Properties of 4-Oxo- and 4-Thio-pyrimido[4,5-*d*]pyrimidine[1]

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4-Aminopyrimidine-5-carboxamide and 4-aminopyrimidine-5-thiocarboxamide reacted with acetic anhydride and triethyl orthoformate to yield 4-oxo- and 4-thiopyrimido[4,5-*d*]pyrimidine, respectively. These compounds showed greater lack of stability to the removal or replacement of substituents than the isomeric pteridines, and were stronger acids and weaker chelating agents.

The considerable antileukemic and carcinostatic activity of several purine derivatives[2] has led to the synthesis of several related heterocyclic systems, most of which were designed in such a way as to permit ribosidation in the equivalent of the 9-position of purines. This seemed to be important since it was believed that 6-mercaptopurine, the purine with the widest use as an antileukemic agent, had to be converted to the riboside or the ribotide before it could become an active metabolite,[3] a view supported by the observation that mercaptopurine-resistant bacteria were incapable of converting hypoxanthine to purine ribotides. Recently, however, it was found that methylation in

(1) This work was presented before the Medicinal Chemistry Section at the American Chemical Society meeting, New York, N. Y., September 1957, 30–O.

(2) J. H. Burchenal in Current Research in Cancer Chemotherapy, Report No. 4, 11 (1956).

(3) H. E. Skipper, J. R. Thomson, D. J. Hutchison, F. M. Schabel, and J. J. Johnson, *Proc. Soc. Expt. Biol. Med.*, **95**, 135 (1957).