it was necessary to prepare radioactively-labeled 6-mercaptopurine for tracer studies. In order to follow the fate of both the purine moiety and of the mercapto group, both the 8-C^{14}- and the 6-S^{35}-labeled compounds were required. The original synthesis of 6-mercaptopurine[6] from hypoxanthine was not considered satisfactory for small quantities of material if the most economical use was to be made of the C^{14}-formate. In the present method, the labeled formate is introduced in the last step.

The method consists of the synthesis of 4,5-diamino-6-mercaptopyrimidine from 4-amino-6-chloro-5-nitropyrimidine[7] followed by formylation and ring closure of the sodium salt of the 5-formyl derivative. For the synthesis of S^{35}-6-mercaptopurine, the reaction of 6-chloropurine with potassium hydrosulfide was employed.

4,5-Diamino-6-mercaptopyrimidine.—A suspension of 15 g. of 4-amino-6-chloro-5-nitropyrimidine[7] in 435 ml. of *N* potassium hydrosulfide solution was heated on the steam-bath for two hours with the intermittent passage of a stream of hydrogen sulfide through the mixture. The solution was cooled, resaturated with hydrogen sulfide and allowed to stand at room temperature overnight. Pale yellow needles separated, were collected by filtration and washed with a small amount of water. The crude precipitate (9 g.) was recrystallized from 350 ml. of water and gave 7.2 g. (60% yield) of colorless 4,5-diamino-6-mercaptopyrimidine after filtration and drying in a vacuum desiccator. An additional 1.2 g. of product was recovered by acidification of the reaction mixture filtrate, removal of the sulfur precipitate, and evaporation of the filtrate to 100 ml. Ultraviolet absorption spectrum: at *p*H 1, λ_{max} 240,305 mμ (E_m 16,100, 17,900); at *p*H 11, λ_{max} 240,309 mμ (E_m 17,100, 15,400).

Anal. Calcd. for $C_4H_6N_4S$: C, 33.8; H, 4.2; N, 39.4. Found: C, 34.1; H, 4.2; N, 39.7.

8-C^{14}-6-Mercaptopurine.—An aqueous solution of C^{14}-sodium formate containing 0.1915 millimole, with an activity of 0.608 millicurie, in 0.574 ml. was evaporated to dryness under reduced pressure at 60° in a flask of 30-ml. capacity. To the residue was added 0.5 g. (3.5 millimoles) of finely powdered 4,5-diamino-6-mercaptopyrimidine and 2.2 ml. of 90% formic acid containing 0.18 millicurie of C^{14}-formic acid. The mixture was heated at 85° for 4.5 hours in the same distillation apparatus used previously for the removal of water. The formic acid was then removed at 50° under reduced pressure and this distillate was stored for reuse. The residue was freed of traces of formic acid by the addition of 3-ml. portions of water three times and the removal of each portion by distillation under reduced pressure.

To the residue was added 3.5 ml. of *N* sodium hydroxide and the mixture was evaporated carefully to dryness at 50° under reduced pressure. The residue was heated gradually to 220° in an oil-bath and the temperature maintained at 220 to 240° for one hour. During this time water was evolved and the melt turned dark orange. After cooling, the residue was dissolved in 70 ml. of water, filtered, acidified with acetic acid to *p*H 5, heated to boiling and filtered hot to remove an amorphous red precipitate. The filtrate was chilled for 24 hours and the yellow crystalline precipitate collected, washed with water and dried in a vacuum desiccator. The yield was 0.38 g. (64%) of 6-mercaptopurine hydrate with an activity of 21.5 microcuries/millimole. Its ultraviolet absorption spectrum was identical with that of analytically pure 6-mercaptopurine.[6]

S^{35}-6-Mercaptopurine.—To 2.5 g. (16.2 millimoles) of powdered 6-chloropurine in a glass bomb was added 1.9 ml. of a solution containing 0.91 mg. per ml. of S^{35}-barium sulfide (with a total radioactivity of 30 millicuries) in 0.188 *N* barium hydroxide, 10 ml. of water and 18 ml. of 2 *N* potassium hydrosulfide solution. The glass tube was sealed and heated in a boiling water-bath for seven hours. A yellow granular precipitate formed. After chilling, the tube was opened and 18 ml. of 2 *N* sodium hydroxide was added. The clear solution was treated with 2 g. of sodium sulfate to precipitate the barium ion. After filtration, the combined filtrates and washings were placed in a 500-ml. flask connected to a series of three vessels containing 25 ml. each of 0.5 *M* cadmium chloride solution and a gas-drying cylinder containing sodium hydroxide pellets. Thirty-seven ml. of 2 *N* hydrochloric acid was run slowly into the flask below the surface of the liquid. A light yellow precipitate began to form immediately. The hydrogen sulfide was driven over by a stream of carbon dioxide for three hours and collected as cadmium sulfide. The gas issuing from the final sodium hydroxide trap was not radioactive. The reaction mixture was filtered and the precipitate washed with water and dried in a vacuum desiccator. The yield was 2.28 g. (83%) with a radioactivity of 595 microcuries/millimole. The ultraviolet absorption spectrum was identical with that of an authentic specimen.

To the filtrate from this first precipitate was added 1 g. of non-radioactive 6-mercaptopurine hydrate dissolved in 4 ml. of 2 *N* sodium hydroxide. The solution was reacidified with 2 *N* hydrochloric acid and the yellow crystalline precipitate collected (0.87 g. with a radioactivity of 110 microcuries/millimole).

THE WELLCOME RESEARCH LABORATORIES
TUCKAHOE, NEW YORK

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Evidence for the Existence of 1-Amino-2-methyl-2-propanol in the Phospholipids of Neurospora

BY GEORGE L. ELLMAN[1] AND HERSCHEL K. MITCHELL

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This investigation, on the lipids of *Neurospora crassa*, provides evidence that the phospholipid fraction contains choline, serine, ethanolamine and, in addition, the base 1-amino-2-methyl-2-propanol. Several other amines were also found to be present in very small concentrations and these have not been identified. That the phospholipid fractions from several animal tissues contain unidentified bases has been observed previously.[2]

It has been found advantageous to prepare dinitrophenyl (DNP) derivatives of crude cephalins obtained by solvent fractionation prior to hydrolysis. The yellow derivatives can then be separated as such by chromatography or the derivatives of the bases can be separated by chromatography after hydrolysis of the cephalins. Details on the latter procedure only are presented here but the former has promise as a method for studying the various components of cephalin fractions. Although considerable quantities of DNP-serine and DNP-ethanolamine were obtained from the Neurospora cephalins only a very small amount of the crystalline derivative that corresponds to that of 1-amino-2-methyl-2-propanol was isolated. Identification of the substance has therefore been dependent on the chromatographic methods, oxidation with periodate and permanganate[3] and melting temperatures.

Experimental

Extraction and Purification of Neurospora Phospholipid.—Wild type *Neurospora crassa* (strain 5256A) was grown in 40-liter carboys for 6–7 days on Fries medium[4] containing 2% sucrose. The mycelia were extracted in batches in the following way: they were collected, pressed as dry as pos-

(1) This paper is based on part of a thesis presented to the Graduate School of the California Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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(3) J. G. Billman, E. E. Parker and W. T. Smith, *ibid.*, **180**, 29 (1949).

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sible by hand, ground in a meat grinder, and suspended in twice their volume (about 400 ml.) of a mixture of ethanol and ethyl ether (3:1) at room temperature. After two days, the suspension was filtered, and the mycelial mass was re-extracted with 200 ml. of the ethanol–ether mixture overnight. The residual mycelia were dried and weighed. The extract was evaporated to about 200 ml. in a flash evaporator, and the aqueous solution so obtained was saturated with sodium chloride and extracted 5–8 times with 50 ml. of chloroform. These chloroform extracts were combined and collected until 1.5 kg. (dry weight) of mycelia had been extracted. After being dried over anhydrous sodium sulfate, the chloroform solution was evaporated (*in vacuo*) to about 350 ml. This solution was poured into 3.5 liters of dry acetone and the mixture was placed in a refrigerator for three days. The solid material was collected by centrifugation, and dissolved in 150 ml. of chloroform. Absolute ethanol (600 ml.) was poured into this solution to precipitate the cephalins, which were removed by centrifugation.

Preparation of Dinitrophenyl Cephalins.—The cephalin fraction was dissolved in 30 ml. of chloroform. To this solution was added 10 ml. of a 7% ethanolic solution of 2,4-dinitrofluorobenzene, and 10 ml. of a 4% aqueous sodium carbonate solution. The mixture was shaken repeatedly. After 20 hours at room temperature, the chloroform layer was removed, and the residual aqueous layer (after being acidified with hydrochloric acid) was extracted with 10 ml. of chloroform. The chloroform solutions were combined and evaporated.

Hydrolysis of the Dinitrophenyl Cephalins.—The dinitrophenyl derivatives from the chloroform solution were suspended in 25 ml. of ligroin (b.p. 80–100°), and 25 ml. of 5 *N* hydrochloric acid. The mixture was heated under reflux for 20 hours. This two phase hydrolysis produces less tarry material than is formed when aqueous acid, or methanolic acid is used. After hydrolysis, the acid layer was extracted twice with 15 ml. of ligroin to remove the fatty acids, evaporated (*in vacuo*) to dryness and finally dried in a desiccator over sodium hydroxide. The residue was stirred with 15 ml. of water and the solution was extracted three times with 10–15 ml. portions of ethyl acetate which removed the colored amine derivatives.

Chromatography of the DNP-Amines. (A) Silicic Acid–Celite Columns.—The adsorbent consisted of two parts (by weight) of Mallinckrodt silicic acid (100 mesh, specially prepared for chromatographic analysis) and one part of Johns-Manville Hyflo Supercel filter aid. The ethyl acetate solution of the DNP-amines was evaporated in the presence of 5 g. of this adsorbent. This material was placed at the top of a 2 cm. × 13 cm. column of the adsorbent. Air pressure was used to force the solvents through the column. The solvents used consisted of ethyl acetate, acetic acid and ligroin in the following proportions: 5:1:94 and 10:2:88. They were used in that order; 75 ml. of each was forced through the column. The first solvent separated the orange band into six components, of which two accounted for most of the color. Each zone was collected in the eluate. After the solvent had been removed the two large fractions and one of the smaller ones crystallized. The second solvent removed most of the remaining orange material in one band. This material also crystallized on removal of the solvent.

(B) On Alumina Impregnated Paper.—Whatman No. 1 paper was impregnated with alumina[5] by the following procedure. A sheet of the paper was placed on a pane of window glass, and the alumina poured onto it. This was then thoroughly rubbed in by hand. Since this procedure is somewhat subjective, known DNP-amines were always run along with the unknowns. Two solvents were used: 10% acetone in ligroin, and 25% 0.1 *N* ammonium hydroxide in propanol.

Identification of Products. (1) Dinitroaniline.—Crystalline material obtained from the first large fraction of the column was recrystallized twice from benzene–ligroin (7:3); yield 14 mg., m.p., 175–176°.

Anal. Calcd. for $C_6H_5N_3O_4$: C, 39.34; H, 2.75; mol. wt., 183. Found: C, 39.41; H, 2.77; mol. wt.,[6] 206 ± 30.

A sample of 2,4-dinitroaniline,[7] m.p., 175–176°, showed no depression in a mixed melting point determination with this crystalline material. Both compounds moved to R_f 0.86 when chromatographed on alumina impregnated paper, using 10% acetone in ligroin as the solvent.

(2) DNP-Ethanolamine.—The largest crystalline fraction from the column was recrystallized from benzene–ligroin (7:3); yield 25 mg., m.p. 82–84°. A mixed melting point determination with DNP-ethanolamine prepared by the method of Porter and Sanger[8] showed no depression. Both materials moved to R_f 0.27 when chromatographed on alumina impregnated paper, using 10% acetone in ligroin as the solvent.

(3) DNP-Serine.—The orange material eluted from the chromatographic column with the second solvent was crystallized from benzene–ligroin (7:3); yield 17 mg., m.p. 196–197°. Mixed melting points determinations with DNP-serine, m.p. 196–197°, showed no depression. Both materials moved to R_f 0.36 when chromatographed on alumina impregnated paper, using a mixture of 1-propanol and 0.1 *N* ammonium hydroxide (3:1) as the solvent.

(4) 1-DNP-Amino-2-methyl-2-propanol.—The only small fraction from the column which crystallized was recrystallized from benzene–ligroin (7:3); yield 12 mg., m.p. 106–108°.

Anal. Calcd. for $C_{10}H_{13}N_3O_5$: C, 47.05; H, 5.13; mol. wt., 255. Found: 47.25; H, 4.86; mol. wt.,[6] 221 ± 35.

The rest of this material (6 mg.) and 2 ml. of concentrated ammonium hydroxide were placed in a glass tube which was sealed and placed in a boiling water-bath for 2 hours.[9] The tube was cooled, opened and placed in a vacuum desiccator over concentrated sulfuric acid. After the aqueous solution had evaporated (4 days), the contents of the tube were dissolved in 2 ml. of 1 *N* hydrochloric acid. This solution was extracted with butanol to remove the dinitrophenol and any unhydrolyzed derivative.

The solution of the amine (1.5 ml.) was neutralized with 0.5 *N* sodium hydroxide, cooled in an ice-bath and 10 ml. of phosphate buffer (*p*H 7.12, 0.1 *M*), 5 ml. of sodium arsenite (0.1 *M*) and 1 ml. of periodic acid (0.1 *M*) were added to it. Nitrogen was bubbled through the solution and the exit gases were led through a trap containing 20 ml. of a 2,4-dinitrophenylhydrazine solution.[10] After 45 minutes the dinitrophenylhydrazine solution was removed and extracted three times with a total of 20 ml. of benzene. This benzene solution was passed through an alumina column (1 cm. × 10 cm.). The excess reagent was adsorbed at the top of the column, and the band of dinitrophenylhydrazones passed through the column and was collected in the eluate. The benzene was removed and the solid recrystallized from 0.5 ml. of ethanol; yield 2 mg., m.p. 124–125°. A mixed melting point with acetone dinitrophenylhydrazone showed no depression. The unknown dinitrophenylhydrazone was also compared with acetone dinitrophenylhydrazone by chromatography on alumina impregnated paper (as described above) using 10% ether (anhydrous) in ligroin as the developing solvent. Both compounds moved the same distance (R_f 0.65).

The periodate reaction mixture was then treated with 2 ml. of concd. sulfuric acid and 10 ml. of dinitrophenylhydrazine solution. After an hour at room temperature, this solution was extracted with three 10-ml. portions of benzene. The benzene solution was poured through an alumina column to remove the dinitrophenylhydrazine. The derivatives were chromatographed on alumina impregnated paper. Two hydrazones were observed. One moved with the R_f of acetone dinitrophenylhydrazone (0.65) and the other moved with the R_f of formaldehyde dinitrophenylhydrazone (0.27). The remainder of the benzene solution was evaporated in the presence of 1.5 g. of alumina. This material was placed on top of a column of alumina, and the column was developed with 10% ether in ligroin. Two colored zones were eluted, and the solvents evaporated therefrom. The first fraction contained acetone dinitrophenylhydrazone. The latter fraction yielded about 1 mg. of orange product, which did not depress the melting point when mixed with authentic formaldehyde dinitrophenylhydrazone (165–166°).

(5) Grade A, Minus 80 mesh alumina, Aluminum Ore Co., E. St. Louis, Ill.

(6) Determined from the absorbence at λ 355 mμ, using ε (molecular extinction coefficient) = 21,000 per dinitrophenyl group, determined from DNP-serine.

(7) Eastman Kodak Co.

(8) R. R. Porter and F. Sanger, *Biochem. J.*, **42**, 287 (1948).

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(10) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 97.

Paper Chromatography of the Amine.—A portion of the amine solution which was obtained by the hydrolysis of the original DNP derivative was chromatographed on Whatman No. 1 filter paper, using butanol saturated with 1% (v./v.) ammonium hydroxide as the developer. Ninhydrin treatment of the chromatograph showed a spot at R_f 0.58. A similar chromatograph was treated with acidified potassium permanganate[9] before development with the solvent. After chromatographic development no ninhydrin reactive material was observed.

Synthesis of 1-Amino-2-methyl-2-propanol.—This compound was prepared from isobutylene oxide and ammonia.[11] The isobutylene oxide was prepared from isobutylene chlorohydrin[12] which was obtained by hydration of methallyl chloride.[13] The latter compound was donated by the Shell Chemicals Corp.

1-Dinitrophenylamino-2-methyl-2-propanol.—This compound was prepared from the amine by the method of Porter and Sanger.[8] The derivative was crystallized twice from benzene–ligroin (7:3). The yellow orange crystals melted at 107–108°.

Comparison of the Isolated and Synthetic Compounds.—A mixture of the isolated and synthetic materials melted at 106–108°. These derivatives were chromatographed on alumina impregnated paper using 10% ether in ligroin as the solvent, both moved to R_f 0.71. The rates of movement on silicic acid–celite columns were the same. The free bases were chromatographed on Whatman No. 1 paper, and both moved with the following R_f's: in butanol saturated with 1% ammonium hydroxide, 0.54; in isobutyric acid saturated with water, 0.87.

(11) T. L. Cairn and J. H. Fletcher, THIS JOURNAL, **63**, 1034 (1941).

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THE KERCKHOFF LABORATORIES OF BIOLOGY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIF.

Reactions of Long-chain Amines. III. Preparation of N,N-Dialkylthiamorpholinium Chlorides[1]

BY JOHN G. ERICKSON

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Lawson and Reid[2] have studied the reactions of mustard gas with several secondary amines in the presence of sodium carbonate. They found that diethylamine, dipropylamine, dibutylamine and piperidine react to form bis-(2-dialkylaminoethyl) sulfides, $(R_2NCH_2CH_2)_2S$. A similar product is apparently formed with dimethylamine but the only products isolated in this case were divinyl sulfide and 2-vinylmercaptoethyldimethylamine, $CH_2{=}CHSCH_2CH_2N(CH_3)_2$. Apparently they obtained no compounds containing thiamorpholine rings.

We have found that long-chain dialkylamines react with mustard gas, forming N,N-dialkylthiamorpholinium chlorides (I)

$$2R_2NH + (ClCH_2CH_2)_2S \longrightarrow R_2NH\cdot HCl + R_2\overset{+}{N}\!<\!\begin{matrix}CH_2CH_2\\CH_2CH_2\end{matrix}\!>\!S \quad Cl^-$$

I

When 2-chloroethyl sulfone is used, the product is the 1,1-dioxide of I. The most suitable reaction conditions appear to require somewhat more than two moles of amine for each mole of mustard gas, the mixtures being heated at about 130°. Compounds similar to I, differing only in the nature of the anion, have been prepared by Niederl, McGreal and Hart,[3] and Hart, McGreal and Camilli.[4] These workers alkylated long-chain N-alkylthiamorpholines with long-chain dialkyl sulfates.

The difference between our results and those of Lawson and Reid is due presumably to the relative success of two competing reactions. The initial reaction products, $R_2NCH_2CH_2SCH_2CH_2Cl$, may cyclize or they may react with a second mole of amine. The long-chain amines are apparently sufficiently unreactive in this case so that the second reaction cannot easily occur and cyclization takes place instead. The reverse is true with the more reactive amines used by Lawson and Reid.

Acknowledgments.—We wish to thank the Chemical Corps for a generous gift of mustard gas. Microanalyses were performed by James Kerns of these laboratories.

Experimental[5]

4,4-Didodecylthiamorpholinium Chloride.—A mixture of didodecylamine (53.0 g., 0.15 mole), mustard gas (90% pure, 10.6 g., 0.06 mole) and butyl alcohol (20 ml.) was heated at 130° for 27 hours. It was then dissolved in hot methanol (300 ml.) and an aqueous solution of 2.5 g. of sodium hydroxide was added. The mixture was chilled and filtered, giving 42.8 g. of unreacted amine. The filtrate was evaporated to dryness and the residue was recrystallized from ethyl acetate to give 5.6 g. (20% yield) of white solid, m.p. 160–200° dec. A second recrystallization gave iridescent white flakes, same m.p.

Anal. Calcd. for $C_{28}H_{58}ClNS$: C, 70.60; H, 12.27; Cl, 7.44; N, 2.94; S, 6.73. Found: C, 71.05; H, 12.50; Cl, 7.55; N, 2.46; S, 6.42.

4,4-Dioctadecylthiamorpholinium Chloride.—A mixture of dioctadecylamine (349.7 g., 0.67 mole), mustard gas (90% pure, 48.2 g., 0.27 mole) and butyl alcohol (25 ml.) was heated at 130–140° for 33 hours. It was poured into hot methanol (2 l.) containing 12.0 g. of sodium hydroxide. The mixture was heated to boiling, cooled, filtered and the filter cake washed with methanol. The combined filtrate and washings were evaporated *in vacuo*. The residue was extracted with hot ethyl acetate (400 ml.) which, after cooling and filtration, gave 41.1 g. of cream-colored product, m.p. 190–198°; m.p., after recrystallization from ethyl acetate, 202–204.5° dec. Further extraction of the crude amine fraction gave an impure product. This was recrystallized from ethyl acetate to give an additional 16.1 g. of product.

Anal. Calcd. for $C_{40}H_{82}ClNS$: C, 74.52; H, 12.82; Cl, 5.50; S, 4.97. Found: C, 74.58; H, 12.65; Cl, 5.32; S, 5.05.

The 1,1-Dioxide of 4,4-Dioctadecylthiamorpholinium Chloride.—This was prepared in two ways. In the first, dioctadecylthiamorpholinium chloride (12.9 g., 0.02 mole) was dissolved in warm (50–60°) acetic acid (60 ml.) and 30% hydrogen peroxide (5.4 g., 0.048 mole) was added dropwise. After 18 hours the mixture was evaporated on the steam-bath and the residue was recrystallized four times from ethyl acetate, giving 6.3 g. (46%) of white solid, m.p. 197–199°.

Anal. Calcd. for $C_{40}H_{82}ClNO_2S$: C, 71.00; H, 12.22; Cl, 5.24; N, 2.07; S, 4.74. Found: C, 71.07; H, 12.63; Cl, 5.17; N, 2.94; S, 5.18.

In the second method, a mixture of dioctadecylamine (62.5 g., 0.12 mole), 2-chloroethyl sulfone (9.5 g., 0.05 mole) and *n*-butyl alcohol (20 ml.) was heated at 130° for 14 hours, mixed with one liter of hot methanol and neutralized with NaOH (2.0 g.). The mixture was cooled, filtered and evaporated to dryness. Recrystallization of the residue

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(5) M.p.'s are corrected.