TABLE I

Alkyl groups	Cyanoacetic esters Yield, %	B. p. °C.	Mm.	4-Iminobarbituric acid Yield, %	M. p.[a] °C.	Oxygen analog M. p., °C.	4-Thiobarbituric acid Yield, %	M. p.[a] °C.	Formula	Nitrogen % Calcd.	Found
Ethyl	93.5	206–209[b]	Atm.								
Ethyl, ethyl	83.5	213–215[c]	Atm.	58.2	293–294[d]	190–191[e,f]	45.8	196–197	$C_8H_{12}O_2N_2S$	13.99	13.84
Ethyl, isopropyl	61.4	222–227[g]	Atm.	45.5	295–296	145[h,f]	30.4	192–193	$C_9H_{14}O_2N_2S$	13.08	12.96
Ethyl, isoamyl	68.54	90–95	1	47.1	293–293.5	153–155[i,f]	[j]				
n-Butyl	79.0	73–77	1								
Ethyl, *n*-butyl	58.69	76–79	1	37.9	285–286	127–127[k,f]	[l]				

[a] All melting points are corrected. [b] Hessler, *Am. Chem. J.*, **22**, 170 (1899), reports 206–211°. [c] Hesse, *ibid.*, **18**, 747 (1896), reports 100–101 at 14 mm. [d] Conrad, *Ann.*, **340**, 317 (1905), reports 295°. [e] Conrad (ref. d) reports 191°. [f] When mixed with an authentic sample prepared in a different manner, the melting point showed no depression. [g] Crossley and LeSueur, *J. Chem. Soc.*, **77**, 91 (1900), reported 226–227° at 756 mm. Fischer, Rode and Brauns, *Ann.*, **402**, 366 (1914), reported 233° at 760 mm. [h] Fischer and Dilthey, *ibid.*, **335**, 346 (1904), report 146°. [i] Shonle and Moment, THIS JOURNAL, **45**, 248 (1923) 150°. [j] A yellow amorphous material was obtained that could not be purified. Oxidation to oxygen analog by Conrad's method produced a compound melting at 153–155°. A mixed melting point showed no depression. [k] Dox and Yoder, THIS JOURNAL, **44**, 1580 (1922), reports 125°. [l] No identifiable product could be obtained.

TABLE II

Alkyl groups	4-Imino-2-thiobarbituric acid Yield, %	M. p., °C.	4-Oxygen analog m. p.,[a] °C.	2-4-Dithiobarbituric acid Yield, %	M. p., °C.	Formula	Nitrogen % Calcd.	Found
Ethyl, ethyl	60.35	255–256[b]	173[c]	47.2	210–210.5[d]	$C_8H_{12}ON_2S_2$	12.95	13.01
Ethyl, isopropyl	44.5	241–242	192[e,f]	31.5	179.5–180.5[g]	$C_9H_{14}ON_2S_2$	12.17	12.21
Ethyl, isoamyl	37.1	260.5–261.5	168[h,f]	36.45	161–162	$C_{11}H_{18}ON_2S_2$	10.84	10.96
Ethyl, *n*-butyl	66.1	263–264	142–144[i,f]	56.75	130.5–131[j]	$C_{10}H_{16}ON_2S_2$	26.24[k]	26.29[k]

[a] All melting points are corrected. [b] Conrad, *Ann.*, **340**, 325 (1905), reports 255°. [c] Miller, Munch, Crossley and Hartung, THIS JOURNAL, **58**, 1090 (1936), report 174.5°. [d] Carrington, *J. Chem. Soc.*, 126 (1944), reports 205–206°. [e] Tabern and Volwiler, THIS JOURNAL, **57**, 1961 (1935), report 192°. [f] When mixed with an authentic sample prepared in a different manner, the melting point showed no depression. [g] Carrington (ref. d) reports 173°. [h] Tabern and Volwiler (ref. e) report 167–169°. [i] Tabern and Volwiler (ref. e) report 144–145°. [j] Carrington (ref. d) reports 127°. [k] Figures represent analyses based on sulfur content.

The 5-ethyl-5-isoamyl-2,4-dithiobarbituric acid showed the most promising indications. It was administered to fifteen rats, only two of which showed signs of twitching and this was very mild. Upon intravenous injection of 20-30 mg. per kg., rabbits slept instantly, recovered the righting reflex in about twelve minutes and the placing reaction in fifteen to twenty minutes. The animals suffered no ill effects; however, 80 mg. per kg. has caused death. A few experiments on cats showed the same effect as in rabbits.

Summary

A method is described wherein, by using hydrogen sulfide under pressure, the imino group of dialkyl-4-iminobarbituric acids or dialkyl-4-imino-2-thiobarbituric acids may be replaced by sulfur.

The preparation of two dialkyl-4-thiobarbituric acids and four dialkyl-2,4-dithiobarbituric acids is reported.

A brief summary of the pharmacological action of these compounds is included.

MINNEAPOLIS, MINNESOTA RECEIVED DECEMBER 3, 1945

[CONTRIBUTION FROM THE LABORATORIES OF WINTHROP CHEMICAL COMPANY, INC.]

The Synthesis of Amino Acids from Ethyl Acetamidomalonate and Ethyl Acetamidocyanoacetate. III. The Use of Primary Halides[1]

BY NOEL F. ALBERTSON

During the last few years there has been a rapid development in the preparation of amino acids by various modifications of the original Sörensen method in which phthalimidomalonic ester is alkylated and degraded to the amino acid. Although aminomalonic ester itself may be used to prepare amino acids,[2] this intermediate has several disadvantages the most important of which is its tendency to undergo N-alkylation as well as C-alkylation. The protection of the amino group by benzoylation enabled Redemann and Dunn[3] to eliminate this disadvantage. The next advance was the use of acetamidomalonic ester.[4] The acetyl group is more readily intro-

(1) For the first two papers in this series see references 4d and 5.

(2) Putochin, *Ber.*, **56**, 2213 (1923); Keimatsu and Kato, *J. Pharm. Soc. Japan*, **49**, 731 (1929); Locquin and Cerchez, *Bull. soc. chim.*, (4) **47**, 1386 (1930).

(3) Redemann and Dunn, *J. Biol. Chem.*, **130**, 341 (1939); see also Painter, THIS JOURNAL, **62**, 232 (1940).

(4) (a) For a summary of literature references see Snyder, Shekleton and Lewis, *ibid.*, **67**, 310 (1945); see also (b) Albertson, Archer and Suter, *ibid.*, **67**, 36 (1945); (c) Howe, Zambito, Snyder and Tishler, *ibid.*, **67**, 38 (1945); (d) Albertson and Archer, *ibid.*, **67**, 308 (1945); (e) Albertson and Archer, *ibid.*, **67**, 2043 (1945).

TABLE I

R		CN RCHNHCOCH₃ COOC₂H₅			COOC₂H₅ RCNHCOCH₃ COOC₂H₅			R CHCOOH NHCOC₆H₅
Alkyl bromide	Yield, %	M. p., °C.	Nitrogen, % Calcd.	Found	M. p., °C.	Nitrogen, % Calcd.	Found	M. p., °C.
Hydrogen	..	130	16.44	[c]	96	6.47	6.44[e]	187[f]
Methyl	71[a]	107	15.21	14.95	90	6.06	6.15	163[g]
Ethyl	85	130	14.14	14.50	83	5.71	5.56	142[h]
Allyl	82	89	13.32	13.12	46	5.44	6.02	109[i]
n-Propyl	70	118	13.22	12.95	94	5.40	5.45[d]	152[k]
β-Methylallyl	82[b]	81	12.49	12.06	93	5.16	5.06[d]	135[l]
n-Butyl	78[a]	90	12.38	12.53	Oil	..	..[d]	136[m]
Isobutyl	65	120	12.38	11.97[d]	84	5.13	5.08[d]	141[n]
n-Amyl	57	80	11.66	12.07	53	4.88	4.88	135[l]
n-Hexyl	81[a]	82	11.02	11.41	49	4.65	4.84	128[l]
n-Heptyl	65	73	10.44	10.29	47	4.44	4.65	128[l]
n-Octyl	81[a]	73	9.92	9.72	49	4.25	4.34	130[l]
n-Nonyl	32	64	9.45	9.76	48	4.08	4.19	124[l]

[a] Iodide employed. [b] Chloride employed. [c] M. p. 129°, see ref. 10. [d] See ref. 6. [e] M. p. 95°, see ref. 10. [f] M. p. 187°; Curtius, *J. prakt. Chem.*, [2] **26**, 171 (1882). [g] M. p. 163°; Fischer, *Ber.*, **32**, 2455 (1899). [h] M. p. 146° cor., Fischer and Mouneyrat, *Ber.*, **33**, 2388 (1900). [i] M. p. 107.5°, Sörensen, *Ber.*, **41**, 3389 (1908). [k] M. p. 152.5°, Slimmer, *Ber.*, **35**, 405 (1902). [l] See Table II. [m] M. p. 134° cor.; Fischer, *Ber.*, **33**, 2382 (1900). [n] M. p. 141° cor., Fischer, *ibid.*, 2374 (1900).

duced and removed than is the benzoyl group and in addition ethyl acetamidomalonate usually gives solid intermediates whereas the alkylated benzamidomalonic esters are oils.[3] Additional advantages have been reported for the use of ethyl acetamidocyanoacetate as a general reagent in amino acid synthesis.[5] However, to date there have not appeared in the literature any experimental details of the use of ethyl acetamidomalonate or ethyl acetamidocyanoacetate for the preparation of amino acids from saturated alkyl halides.

During the course of investigations conducted in these laboratories, a number of amino acids and their intermediates have been prepared most of which have not previously been described.[6] Both ethyl acetamidomalonate and ethyl acetamidocyanoacetate were alkylated with all of the saturated normal halides up to nonyl. In addition, allyl bromide, β-methylallyl chloride and isobutyl bromide were used as alkylating agents. The intermediates were all hydrolyzed to the amino acids. Since decomposition points are usually less characteristic than melting points, the amino acids were converted to the N-benzoyl derivatives by the method of Carter and Stevens.[7] The yields were generally excellent. It was observed that the time for benzoylation decreased as the chain length of the amino acid increased.

Table I gives the melting points and the nitrogen analyses of the intermediates. In addition, there are included the melting points of the benzoyl derivatives of the corresponding amino acids and the yields obtained on alkylation of acetamidocyanoacetic ester. Table II gives yields, melting points and analyses of some new amino acids. These amino acids are soluble in glacial acetic acid, but except for methylallylglycine are rather insoluble in water. For example, it required about 60 ml. per gram to recrystallize α-aminoheptanoic acid from water.

TABLE II

Amino acid	Yield, %	M. p., °C.	Amino acid nitrogen, % Calcd.	Found	N-Benzoyl nitrogen, % Calcd.	Found
Methylallylglycine	63	215	10.84	10.60	6.00	5.82
α-Aminoheptanoic	55	281	9.64	9.54	5.61	5.39
α-Aminooctanoic	82	270	8.80	8.90	5.32	5.26
α-Aminononanoic	55	273	8.08	7.83	5.05	4.84
α-Aminodecanoic	67	264	7.48	7.45	4.81	4.57
α-Aminoundecanoic	50	253	6.96	6.58	4.59	4.51

In general, alkylation was effected by refluxing in absolute alcohol the sodium salt of ethyl acetamidomalonate or ethyl acetamidocyanoacetate with a five to ten per cent. excess of alkyl halide until the solution was neutral to litmus. The presence of even small percentages of water resulted in decreased yields. The products were isolated by methods illustrated in the experimental section.

Intermediates obtained from ethyl acetamidomalonate were hydrolyzed to the amino acid by boiling with 48% hydrobromic acid or 37% hydrochloric acid for two to eight hours. Intermediates obtained from ethyl acetamidocyanoacetate were hydrolyzed with either acids or by refluxing with 10 to 15% sodium hydroxide for

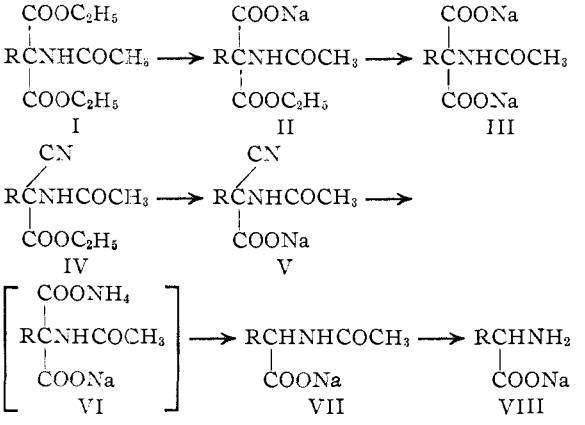
(5) Albertson and Tullar, THIS JOURNAL, **67**, 502 (1945).

(6) Three compounds (the *n*-propyl, *n*-butyl and isobutyl acetamidomalonic esters) have been described in the literature (ref. 4a). They were hydrolyzed to the acetyl derivatives of the corresponding amino acids, but not to the free amino acids. The β-methylallyl and isobutyl acetamidomalonic esters (ref. 4d) and isobutyl acetamidocyanoacetic ester (ref. 5) have also been previously reported.

(7) Carter and Stevens, *J. Biol. Chem.*, **138**, 627 (1941).

about sixteen hours. The acid method of hydrolysis is probably preferable (except for the preparation of γ-methylallylglycine) since it is much more rapid and does not lead to the formation of silica. No difference has been found in the results obtained with hydrobromic or hydrochloric acids. It is probable that acid hydrolysis is complete within two or three hours, since the same yield of amino acid has been obtained by two-hour and by eight-hour hydrolysis. In contrast, the evolution of ammonia during basic hydrolysis may frequently be detected for fifteen hours or more. The yields on hydrolysis could undoubtedly be improved in most cases.

The difference in behavior of the two types of intermediates on basic hydrolysis is interesting.



Treatment of diethyl alkylacetamidomalonate (I) with one equivalent of base gives primarily compound II. Additional base converts II to III. However, prolonged boiling of III with excess base causes no further hydrolysis.[8] In a similar manner, one equivalent of base converts ethyl alkylacetamidocyanoacetate (IV) to V. Acetamidocyanoacetic ester itself, for example, gives acetamidocyanoacetic acid in 80% yield. Treatment with more alkali, however, results in the liberation of ammonia and the formation of a carbonate in solution, indicating that carbon dioxide is also evolved. All attempts to isolate an acetamidomalonic acid have been unsuccessful. The next compound which has been isolated is the acetylated amino acid corresponding to the sodium salt VII. This is readily hydrolyzed by further boiling with base to give VIII. The hydrolysis probably proceeds through the intermediate VI; it cannot go from V to III or one would obtain III as the end product. A compound of type VI would undoubtedly be unstable in solution and rapidly be converted to VII by loss of ammonia and carbon dioxide.[9]

A comparison of the reduction of the respective allyl condensation products to compounds identical with the *n*-propyl derivatives indicates one advantage of acetamidomalonic ester over acetamidocyanoacetic ester. In the former instance the double bond is reduced rapidly and quantitatively; in the latter instance, even with a palladium catalyst the reduction is slow and a considerable reduction of the nitrile group apparently occurs before the double bond is reduced.

The details of representative procedures are described in the experimental section.

(8) *Cf.* Snyder and Smith, THIS JOURNAL, **66**, 350 (1944).

(9) In connection with another problem it was discovered that the ammonium salt of 3-acetamido-3-carboxy-α-piperidone lost ammonia and carbon dioxide merely on recrystallization from warm water to give 3-acetamido-α-piperidone.

Experimental

Ethyl *n*-Butylacetamidocyanoacetate.—To a solution of 2.3 g. of sodium in 50 ml. of absolute ethanol there was added 17.0 g. of ethyl acetamidocyanoacetate,[10] followed by 12.0 ml. of *n*-butyl iodide. The solution was refluxed overnight and poured onto ice and water. The solid thus obtained was filtered and dried, yielding 20.5 g. (91%) of crude product, m. p. 68–75°. Recrystallization from aqueous ethanol gave 17.6 g. (78%) of needles, m. p. 87–89°. An analytical sample melted at 90°.

This procedure is applicable to all members of the acetamidocyanoacetic ester series, the compounds of which are less soluble in water than the acetamidomalonic ester derivatives.

Diethyl Methylacetamidomalonate.—Five milliliters of methyl iodide was added to a solution of 1.15 g. of sodium and 10.9 g. of acetamidomalonic ester[10,11] in 25 ml. of ethanol and refluxed for thirteen hours. The alcohol was then removed *in vacuo* and the yellow residue was dissolved in 20 ml. of water containing at least sufficient acetic acid to neutralize any unreacted base. The solution was cooled and the product was filtered and washed successively with 10-ml. and 5-ml. portions of ice water to remove the yellow color. The dry product weighed 10.2 g. (88%) and melted at 85–88°. Recrystallization of this material from water, with but little loss, raised the m. p. to 88–90°.

The use of dimethyl sulfate as an alkylating agent gave an 80 per cent. yield of crude product melting at 70–76°, but further purification was difficult, possibly because of transesterification.

In the case of allyl bromide and *n*-butyl bromide the products of condensation with acetamidomalonic ester obtained by the above procedure were oils. The oily products were separated from water by extraction with chloroform and the chloroform removed *in vacuo*. The viscous residues were dissolved in a small amount of benzene and petroleum ether was added. This procedure caused the allylacetamidomalonic ester to crystallize, but the *n*-butyl derivative remained an oil (*cf.* ref. 4a).

The higher members of the series (isobutylacetamidomalonic ester and above) are sufficiently water insoluble to be isolated by pouring onto ice and water like the acetamidocyanoacetic esters.

Basic Hydrolysis: *dl*-γ-Methylallylglycine.—Six and three-tenths grams of ethyl methylallylacetamidocyanoacetate was refluxed for twenty hours with 80 ml. of 10% sodium hydroxide solution. The solution was acidified with 20 ml. of cold concentrated hydrochloric acid and concentrated to dryness *in vacuo*. Absolute alcohol was added and the mixture was concentrated to remove all traces of water. The residue was then extracted with absolute ethanol and the amino acid was precipitated from solution by the addition of excess pyridine. The mixture was cooled overnight and the product filtered, washed with ethanol and dried to yield 2.3 g. (63%) of amino acid, m. p. 201–205°. Purification was effected by dissolving the product in water (10 ml. per g.), treating with charcoal, cooling and adding an equal volume of 95% alcohol. The shiny platelets thus obtained melted at 214–215° with gas evolution.

(10) Cerchez and Colesiu, *Compt. rend.*, **194**, 1954 (1932).

(11) Snyder and Smith, THIS JOURNAL, **66**, 351 (1944).

Anal. Calcd. for $C_6H_{11}O_2N$: N, 10.84. Found: N, 10.60.

Acid Hydrolysis: (A) *dl*-α-Aminononanoic Acid.—A solution of 22.2 g. of diethyl *n*-heptylacetamidomalonate in 100 ml. of concentrated hydrochloric acid was refluxed for seven hours and then cooled to 0°. There was thus precipitated a mass of shiny pearly plates. This material was collected by filtration and dried yielding 10.7 g. of amino acid hydrochloride. The crude product was dissolved in a mixture of ethanol and water (1:1) and the amino acid precipitated by the addition of 15 ml. of pyridine. The product was filtered, washed and air dried; yield 6.7 g. (55%). It was further purified with little or no loss by solution in 500 ml. of water containing 15 ml. of 10% sodium hydroxide, treating with charcoal, filtering and adding excess acetic acid to precipitate the amino acid. The pure compound melted at 270–273° (dec.).

Anal. Calcd. for $C_9H_{19}O_2N$: N, 8.08. Found: N, 7.83.

This amino acid could be isolated equally well by the use of ammonium hydroxide in place of pyridine since it is not very soluble in water. (Compare the isolation of *dl*-leucine.[4d]) Procedure (B) is preferable for lower molecular weight amino acids.

Acid Hydrolysis: (B) *dl*-α-Aminobutyric Acid.—A solution of 9.9 g. of ethyl ethylacetamidocyanoacetate in 40 ml. of 40% hydrobromic acid was refluxed for eight hours, treated with charcoal, cooled, filtered and concentrated *in vacuo* to dryness. The residue was extracted with four 10-ml. portions of absolute ethanol, diluted with ethanol and adjusted to *p*H 6 by addition of pyridine. There was thus obtained 4.24 g. (82.4%) of *dl*-α-aminobutyric acid identified by means of its benzoyl (Table I) and chloroacetyl derivatives.

Reduction of Ethyl Allylacetamidocyanoacetate.—To a solution of 6.3 g. of ethyl allylacetamidocyanoacetate (prepared by the method outlined for ethyl *n*-butylacetamidocyanoacetate) in 100 ml. of ethanol there was added 0.100 g. of palladium chloride and one gram of Nuchar. It required about one and one-half hours at room temperature and 40 pounds pressure to take up one equivalent of hydrogen. The reduction was then stopped and the catalyst and solvent were removed. The residue was dissolved in chloroform, washed with dilute hydrochloric acid and then with water, and the chloroform removed *in vacuo*. The residue was taken up in ethanol, treated with charcoal, filtered and diluted with water. Cooling gave 2.5 g. of white platelets melting at 76–79°. After two recrystallizations from water the product was shown by melting point and mixed melting point to be identical with ethyl *n*-propylacetamidocyanoacetate obtained from *n*-propyl bromide and acetamidocyanoacetic ester (Table I).

Reduction of Diethyl Allylacetamidomalonate.—Reduction of a solution of 2.2 g. of diethyl allylacetamidomalonate (see above) in 100 ml. of ethanol using Raney nickel catalyst was complete in one minute at room temperature and 40 pounds pressure. Upon removal of the catalyst and solvent the residue crystallized with the liberation of heat. The yield of product melting at 91–94° was quantitative. When mixed with diethyl *n*-propylacetamidomalonate, m. p. 93–95° (prepared from *n*-propyl bromide and acetamidomalonic ester by the method given for methyl iodide) the mixture melted at 93–94°.

Acetamidocyanoacetic Acid.—Seventeen grams of ethyl acetamidocyanoacetate was added to a solution of 5.6 g. of potassium hydroxide in 50 ml. of water and 100 ml. of ethanol and allowed to stand for forty-eight hours at room temperature. Most of the solvent was then removed *in vacuo*, and 50 ml. of water was added to the residue. Chloroform was added to dissolve the unreacted ester and the water layer was extracted with chloroform. Evaporation of the chloroform gave 8.0 g. of recovered acetamidocyanoacetic ester. The aqueous layer was cooled and acidified by the slow addition of concentrated hydrochloric acid to precipitate the product. The product was filtered and washed with ice water to give 6.0 g. of acetamidocyanoacetic acid, m. p. 117–118.5°. The yield was 80% based on the amount of acetamidocyanoacetic ester used. An analytical sample melted at 119–120.5°. The product evolved ammonia on warming with sodium hydroxide and lost carbon dioxide on heating an aqueous solution.

Anal. Calcd. for $C_5H_6O_3N_2$: N, 19.72. Found: N, 20.15.

Acknowledgment.—The author is indebted to Miss Audrey Fiescher and Mrs. Ruth Bachand for technical assistance, and to Miss Alice Rainey and Mr. Morris Auerbach for the nitrogen analyses.

Summary

Diethyl acetamidomalonate and ethyl acetamidocyanoacetate have been alkylated with all of the normal saturated alkyl halides up to and including nonyl, as well as with allyl, β-methylallyl and isobutyl halides. The resulting products have been hydrolyzed to amino acids which have been characterized through the benzoyl derivatives.

RENSSELAER, N. Y. RECEIVED OCTOBER 29, 1945

[CONTRIBUTION FROM THE CHEMOTHERAPY DIVISION, STAMFORD RESEARCH LABORATORIES AND THE CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Studies in Chemotherapy. XIII. Antimalarials. Halogenated Sulfanilamidoheterocycles

BY J. P. ENGLISH, J. H. CLARK, J. W. CLAPP, DORIS SEEGER AND R. H. EBEL

In the course of the testing of sulfanilamides against sporozoite-induced infections of *Plasmodium gallinaceum* in the chicken it was found[1] that 2-sulfanilamido-5-bromopyrimidine and its chlorine analog showed an unusual property. This was demonstrated when an attempt was made to counteract the activity of these compounds by *p*-aminobenzoic acid which had previously prevented the activity of all the sulfanilamides against which it had been tested.[1,2] Contrary to expectations, the activity of these halogenated sulfadiazines was only partially reduced, rather than completely abolished, by *p*-aminobenzoic acid.

This unexpected observation led to the prepara-

(1) Special aspects of this subject will be discussed by Drs. S. Brackett and E. Waletzky of this Laboratory in a paper which is now in preparation. The routine testing of these compounds will be reported under Test 0-2 in the forthcoming monograph, "A Review of Antimalarial Drugs, 1941–1945," F. Y. Wiselogle, *Editor*.

(2) Marshall, Litchfield and White, *J. Pharmacol.*, **75**, 89 (1942); Seeler, Graessle and Dusenberry, *J. Bact.*, **45**, 205 (1943); Maier and Riley, *Proc. Soc. Exptl. Biol. Med.*, **50**, 152 (1942).