A Methionine Substitute: 4-Methylthiobutane-1,2-diol

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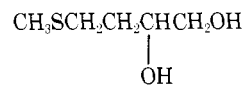
4-Methylthiobutane-1,2-diol (1), a new compound, is fully equivalent to methionine on a weight basis in supporting the growth of young rats fed a diet of crystalline amino acids, but it is only about 60–70% as effective as methionine in supporting the growth of fast growing young broilers. Several new compounds structurally related to the diol also tested in broiler diets were not effective. The results from subcutaneous injection of the diol 1 into chicks suggested that chicks are unable to convert it to methionine or methionine hydroxy analog with high efficiency. Demonstration of unchanged diol 1 in the chick droppings indicated that incomplete absorption from the gut is also of some importance in limiting its utilization. 4-Methylthiobutane-1,2-diol (1), bp 114°C (0.3 mm), was made by both the addition of methyl mercaptan to 3-butene-1,2-diol (76% conversion, 97% yield) and lithium aluminum hydride ($LiAlH_4$) reduction of ethyl 2-hydroxy-4-methylthiobutyrate. The L-diol 1, $[\alpha]^{25}D$ −41.4° (*c* 2.5, MeOH), was made from L-methionine as starting material. The D-diol 1 was obtained 90% optically pure, $[\alpha]^{25}D$ +37° (*c* 2.5, MeOH), by resolution of the bisphthalate half-ester. Esters of the diol 1 were synthesized: diacetate, bp 89°C (0.25 mm), 67.4% yield; mixture of linoleates, bp 165–185°C (3×10^{-4} mm); succinate, as a residue not well characterized. Oxidation with Cr_2O_3 of a mixture of monoacetates of the diol gave a mixture of 2-keto-3-butenyl acetate and 2-acetoxy-3-butenol. A base-catalyzed reaction of methyl mercaptan with the mixture gave 2-keto-4-thiomethylbutyl acetate. 4-Thiomethyl-2-butanone, bp 29–30°C (0.5 mm), and 4-thiomethyl-2-butanol, bp 46–47°C (0.6 mm), were also synthesized. Other new compounds obtained were 3-methylthiobutane-1,2-diol, 2-amino-4-methylthiobutanol, 4-thiomethyl-2-butanone, and 4-thiomethyl-2-butanol.

The growth of rats on a diet deficient in methionine has been shown to be supported by DL-2-hydroxy-4-methylthiobutyric acid (methionine hydroxy analog or MHA) (Block and Jackson, 1932; Jackson and Block, 1933). This is the only known instance in which an amino acid has been successfully replaced by an analog. That example stimulated us to conceive the possibility that a hitherto unknown compound, 4-methylthiobutane-1,2-diol (1), could also be



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metabolized to methionine. This concept is supported by several literature references to the assimilation, toxicology, and metabolism of various vicinal glycols (Bayley et al., 1967; Bowen and Waldroup, 1969; Hanzlik et al., 1939a,b; Huff, 1961; Laug et al., 1939; Persons et al., 1968; Rudney, 1954; Scholz, 1950; Waldroup and Bowen, 1968; Yoshida et al., 1969; Yoshida and Ikumo, 1971). Among these references, particularly noteworthy is the comment by Laug et al. (1939) that 1,2-propanediol may be converted into a normal body constituent. This comment is at odds, however, with the results reported by Yoshida and Ikumo (1971) who demonstrated that chickens receiving about 5% of 1,2-propanediol excreted significant amounts of propionic acid. Their experiments do not support our working hypothesis, which predicated that the diol 1 might be converted in animals by normal metabolic oxidation of the primary hydroxyl group to a carboxyl group, resulting in the transformation of compound 1 to methionine hydroxy analog. A further precedent for this possibility exists in the biological transformation of pantothenol (2,4-dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethylbutyramide) in warm-blooded animals to pantothenic acid, as reported by Burlet (1944).

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EXPERIMENTAL SECTION

Materials. 4-Methylthiobutane-1,2-diol and its derivatives, 4-methylthio-2-butanone, 4-methylthio-2-butanol, and 2-amino-4-methylthiobutanol, were synthesized as described herewith.

Other chemicals were purchased from the Aldrich Chemical Co., Inc.: DL-homocysteine thiolactone hydrochloride (H1586-2); DL-methionine (M885-1); from Pfaltz and Bauer, Inc.: DL-homocystine (H07420) and DL-homocysteine (H07360); and from Badische Anilin und Soda Fabrik, Ludwigshafen, West Germany: 3-butene-1,2-diol. For the rat feeding diets, two components were purchased from General Biochemicals, Chagrin Falls, Ohio: Catalog No. 40055 Vitamin Mix A.O.A.C. and Catalog No. 170760 Rogers-Harper Salt Mix.

4-Methylthiobutane-1,2-diol. A mixture of 880 g (10 mol) of 3-butene-1,2-diol (2) and 570 g (11.9 mol) of methyl mercaptan was placed in a 2-l. stirred autoclave arranged for periodic addition of 0.35 ml of a 25% solution of *tert*-butyl perphthalate dissolved in butyl phthalate. With the reaction mixture at 22°C, doses of the perester solution were added the first two times at 4-min intervals and then 22 times over a period of about 5.5 hr at intervals of about 15 min. The temperature of the reaction mixture rose gradually in the first 3 hr to 44°C and during the next hour was warmed by a heating jacket to about 70°C. The reaction mixture reached a maximum temperature of 74°C, and the maximum pressure in the autoclave was 45 psig (at the end of the reaction). A total of 8.4 ml of perester solution was used. The stirring and heating were stopped and the autoclave allowed to cool to 25°C. The excess methyl mercaptan was vented, using a 10% solution of sodium hydroxide to absorb it, and the reaction product then was subjected to a 30–40 mm vacuum in a rotary evaporator at 30–35°C. Distillation through a short column gave a main fraction of 812.5 g (7.24 mol) of the desired product, bp 114°C (0.3 mm). A forerun of 178.6 g (2.03 mol) of compound 2 was collected at 40–50°C (0.4 mm). The conversion to compound 1 was 75.9% and the yield was 96.6%. The infrared and NMR spectra for the product were consistent with the structure proposed. The mass spectrum showed a molecular ion at *m/e* 136.

The quality of the starting material **2** was most conveniently assessed by GLC using a 6 ft × 0.25 in. column filled with 20% SE 30 on Diatoport, with temperature programming raising the operating temperature from 50 to 250°C at 15°C/min. The diol **2** was eluted when the column temperature reached about 128°C. The purity of diol **1** was also ascertained by GLC, using a 2 ft × 0.25 in. column filled with 5% Carbowax 20M on Diatoport and a temperature program which raised the column temperature from 50 to 300°C at the rate of 15°C/min. Diol **1** was eluted at about 250°C.

3-Methylthiobutane-1,2-diol. A low boiling fraction (bp 83–98°C (0.5 mm)) from the distillation of the diol **1** (prepared from compound **2** and methyl mercaptan) was subjected to GLC. Two major components were present. Sufficient quantities of each were collected by repeated GLC injections to allow identification by mass spectroscopy. Each compound has a parent peak at *m/e* 136 and the fragmentation pattern confirms the structures.

Ethyl Ester of MHA. In a 12-l. round-bottomed flask fitted with a thermometer, dropping funnel, stirrer, reflux condenser, and provision for passing in a stream of dry nitrogen was placed 2 l. of absolute ethanol and 1535 g (9.08 mol) of the calcium salt of MHA. Over a period of 45 min, a solution of 2.5 l. of absolute alcohol containing 34 g (9.6 mol) of hydrogen chloride was added to the reaction vessel. The mixture was heated to the boiling point over a 45-min period and refluxed for 20 min. Then about 4 l. of ethanol was removed from the reaction mixture by distillation. The residue was diluted with 1 l. of ethylene dichloride and then extracted once with 3 l. of water. The volatile materials were removed from the organic layer by distillation and the residue distilled under diminished pressure to give 1395.4 g (7.83 mol) or an 86.3% yield of the ethyl ester of MHA. The NMR spectrum of the ester was in agreement with its structure.

$LiAlH_4$ Reduction of MHA Ester. In a 22-l. round-bottomed flask fitted with a thermometer, dropping funnel, stirrer, reflux condenser, and an inlet for dry nitrogen was placed 4 l. of tetrahydrofuran. To this solvent 214 g (5.64 mol) of $LiAlH_4$ was added over a 20-min period. During this operation, the mixture warmed to 49°C. It was cooled to 32°C and 1190 g (6.69 mol) of MHA ester dissolved in 2 l. of tetrahydrofuran was added over a period of 90 min with cooling of the reaction mixture in an ice bath to keep the temperature at about 30–40°C. When all of the ester had been added, the reaction mixture was heated to the boiling point and maintained there for 105 min. It was cooled to 30°C and 214 ml of water was added cautiously. The mixture was stirred for 10 min after the water had been added, and a solution of 193 ml of 50% sodium hydroxide solution added followed by 450 ml of water and 2 l. of tetrahydrofuran. The reaction mass was heated to the boiling point and 215 ml of water added slowly. The mixture was cooled and filtered and the filter cake washed with tetrahydrofuran. The tetrahydrofuran was removed from the filtrate and washings by distillation and combined with the similar products from two other identical experiments. Distillation of the nonvolatile residues gave 2323 g of the diol **1**. The product was identical with that obtained by addition of methyl mercaptan to 3-butene-1,2-diol.

L-4-Methylthiobutane-1,2-diol. A solution of 50 g of sodium nitrite in 100 ml of water at 0°C was added over a period of 1 hr to an ice-cold solution of 100 g of L-methionine dissolved in a mixture of 450 ml of 1.5 *N* sulfuric acid and 300 ml of water. The reaction mixture was allowed to stand for 30 min at 0°C and 20 g of urea then added, followed by 60 ml of 12 *N* hydrochloric acid. The mixture was freed of nitrogen oxides by distilling about one-third of the initial volume under diminished pressure at 25°C. The residue was extracted five times with 500-ml portions of ether. After the extract had been dried over anhydrous magnesium sulfate, the ether was evaporated giving a residue of 13.5 g of crude L-2-hydroxy-4-methylthiobutyric acid.

In a 1000-ml round-bottomed flask fitted with a reflux condenser, mechanical stirrer, separatory funnel, and inlet for dry nitrogen was placed 10 g of lithium aluminum hydride and 200 ml of dry tetrahydrofuran. To this suspension was added at 25°C a solution of 13.4 g of the previously prepared L-2-hydroxy-4-methylthiobutyric acid. When the addition had been completed, the mixture was refluxed for 4.5 hr, cooled to 10°C, and the following solutions were added successively: (1) 10 ml of water in 30 ml of tetrahydrofuran, (2) 10 ml of 15% aqueous sodium hydroxide, and (3) 30 ml of water in 30 ml of tetrahydrofuran. The reaction mixture was then filtered and the filter cake washed with three 50-ml portions of tetrahydrofuran. The combined filtrate was distilled under atmospheric pressure to remove the tetrahydrofuran and the remaining residue distilled under diminished pressure to yield 10 g of L-4-methylthiobutane-1,2-diol, bp 100–102.5°C (0.2 mm), $[\alpha]^{25}D$ −41.4° (*c* 2.5, MeOH). The infrared and 60-MHz NMR spectra for the material were identical with those for DL-methylthiobutane-1,2-diol (**1**).

Anal. Calcd for $C_5H_{12}O_2S$: C, 44.1; H, 8.83; S, 23.55. Found: C, 44.09; H, 8.86; S, 23.19.

Resolution of DL-4-Methylthiobutane-1,2-diol. A mixture of 100 g (0.73 mol) of the diol **1**, 240 g (1.6 mol) of phthalic anhydride, and 500 ml of dry pyridine in a 2-l. round-bottomed flask was boiled for 5 hr under a reflux condenser. The pyridine was distilled from the reaction mixture, the residue dissolved in 1 l. of chloroform, and the solution extracted with 1 l. of 1 *N* sodium hydroxide solution. The aqueous extract was acidified with 12 *N* hydrochloric acid to pH 2 and extracted with two 900-ml portions of chloroform. This extract was washed with 200 ml of water and dried over anhydrous magnesium sulfate and the chloroform removed by distillation. The nonvolatile residue from the distillation was crystallized from 500 ml of benzene to give 151 g of crystals of bisphthalate half-ester of **1**, mp 105–107°C. A second crop amounting to 23.3 g was obtained from the mother liquors for a total yield of 83.5%.

To a mixture of 102 g (0.235 mol) of the bisphthalate half-ester with 300 ml of toluene was added a solution of 57 g (0.423 mol) of L-amphetamine in 50 ml of toluene. The resulting mixture was heated to 60°C and filtered and the filtrate allowed to stand at 20–25°C for 3 hr. Crystals of the D-4-methylthiobutane-1,2-diol bisphthalate salt of L-amphetamine which formed were filtered and washed with cold toluene. The yield was 75.7 g, corresponding to 45.5% of the theoretical amount, $[\alpha]^{25}D$ +3.50° (*c* 4, methanol). Recrystallization of this salt from 750 ml of toluene gave product with $[\alpha]^{25}D$ +7.18° (*c* 4, MeOH). These crystals were dissolved in a mixture of 400 ml of ether and 250 ml of 1 *N* hydrochloric acid. The ether layer was separated and washed with 100 ml of water and the ether removed by distillation. The residue was mixed with 300 ml of 1 *N* sodium hydroxide solution and the mixture boiled under a reflux condenser for 5 min. The excess sodium hydroxide was neutralized with carbon dioxide gas and most of the water removed from the solution by distillation under diminished pressure to give 5.5 g (0.041 mol) of D-4-methylthiobutane-1,2-diol; $[\alpha]^{25}D$ +29.2° (*c* 2.5, MeOH), which corresponds to an optical purity of 71%. A sample of D-4-methylthiobutane-1,2-diol with $[\alpha]^{25}D$ +37°, or 90% optically pure, was obtained after recrystallizations from toluene of the D-4-methylthiobutane-1,2-diol bisphthalate salt of L-amphetamine, followed by acid decomposition and saponification.

2-Amino-4-methylthiobutanol. In a 3-l. round-bottomed flask fitted with a stirrer, thermometer, reflux condenser, and provision for flushing with a stream of dry nitrogen was placed 1 l. of tetrahydrofuran and 37 g (1 mol) of $LiAlH_4$. The mixture was cooled to 20°C and 149 g of DL-methionine added over a period of 1 hr with cooling by

an ice bath during this time to maintain the reaction temperature at 20–24°C. The reaction mixture soon became so viscous that it was necessary to add 1.5 l. of tetrahydrofuran to permit stirring. The reaction mixture was boiled for 45 min and cooled to 25°C. Then in succession there was added to the reaction mixture 37 ml of water, 110 ml of 15% sodium hydroxide solution, and, finally, 37 ml of water. This mixture was stirred and heated to 50°C for 10 min, cooled, and filtered. The filter cake was washed with tetrahydrofuran and the total filtrate distilled to remove the tetrahydrofuran. The residue on distillation gave a main fraction of 2-amino-4-methylthiobutanol, bp 119° (1.0 mm), weighing 47.7 g, corresponding to a 35.3% yield.

The mass spectrum of the compound showed a molecular ion of *m/e* 135 in agreement with the theoretical value. Both the 60- and 100-MHz NMR spectra agreed with the predicted structure.

4-Methylthiobutane-1,2-diol Diacetate. A mixture of 68 g (0.5 mol) of the diol 1, 112 g (1.1 mol) of acetic anhydride, and 1 g of zinc chloride was heated for 3 hr on a steam bath, cooled to 25°C, and then poured into 150 ml of cold water. The reaction mixture was extracted with three 100-ml portions of benzene and the extract washed once with 100 ml of water. After the benzene solution had been dried with anhydrous magnesium sulfate, the solvent was removed by distillation under atmospheric pressure and the residue distilled to give a main fraction, bp 89°C (0.25 mm) and weighing 75.4 g. This corresponds to a yield of 67.4% of the desired diacetate. The 60-MHz NMR spectrum of the product agreed with the postulated structure and the infrared spectrum further confirmed its identity.

4-Methylthiobutane-1,2-diol Linoleates. In a 1-l. round-bottomed flask fitted with a condenser, thermometer, stirrer, and dropping funnel was placed a mixture of 45 g (0.33 mol) of the diol 1, 52.7 g (0.67 mol) of dry pyridine, and 200 ml of anhydrous ether. Beginning with the mixture at 25°C, 201.8 g of linoleyl chloride was added over a 2-hr period. The temperature of the reaction increased so that the ether boiled. When the addition of the acid chloride had been completed, the reaction mixture was boiled for another hour. It was then allowed to cool and stand at room temperature for 16 hr. The reaction mixture was then filtered, the filter cake washed with ether, and the filtrate freed of ether by distillation at atmospheric pressure. The residue was distilled to give 73 g of a material, bp 165–185°C (3×10^{-4} mm). The mass spectrum of this distillate showed no molecular ion, but the fragmentation pattern was consistent with that expected for a mixture of mono esters. The 100-MHz NMR spectrum confirmed the postulated structure and also indicated the presence of both dilinoleate ester and free linoleic acid. The residue from the distillation on examination by mass spectroscopy showed the expected molecular ion for the dilinoleate ester. The 100-MHz NMR spectrum for the material was in good agreement with the dilinoleate structure, and its infrared spectrum showed no hydroxyl absorption band.

4-Methylthiobutane-1,2-diol Succinate Ester. A mixture of 100 g (1.0 mol) of succinic anhydride, 136 g (1.0 mol) of compound 1, 100 ml of benzene, and 0.5 g of zinc chloride was boiled under a reflux condenser for 1 hr. The reaction mixture was allowed to cool and let stand at room temperature for 64 hr. It was diluted with 100 ml of benzene and extracted with two 500-ml portions of water. The water was evaporated from these aqueous extracts under diminished pressure in a rotary evaporator with a water bath at a temperature not exceeding 90°C. The residue was dried by azeotropic distillation with benzene. Examination of the residue by mass spectroscopy gave a fragmentation pattern consistent with that for the molecular ion less 17 (OH). The 100-MHz NMR spectrum for the material was largely in agreement with the proposed structure. Minor discrepancies were ascribed to impurities.

Table I. Cumulative Average Body Weight Gains for Rats, Amino Acid Diet (9-Day Test)

Diet[a]	Av cumulative wt gain, g, at day 2	4	6	8	9
Basal	−3.2	−4.1	−4.9	−5.5	−5.8
Basal + DL-methylthiobutane-1,2-diol	10.0	23.2	32.8	43.1	47.0
Basal + DL-methionine	6.0	10.5	27.4	32.6	37.4

[a] Each group consisted of ten male weanling albino rats.

Table II. Cumulative Average Body Weight Gains for Rats, Amino Acid Diet (25-Day Test)

Diet[a]	Av cumulative wt gain, g, at day 3	7	11	18	25
Basal	−7.0	−9.2	−11.3	−7.5	−8.0
Basal + DL-methiobutane-1,2-diol	13.0	27.2	38.9	67.0	102.8
Basal + DL-methionine	14.3	30.2	42.4	66.9	103.6

[a] Each group consisted of ten male weanling rats.

2-Keto-4-methylthiobutyl Acetate. Analysis of a mixture of 3-butene-1,2-diol (2) with its monoacetates (Dr. Samuel Tinsley, Union Carbide Corporation) by GLC on a 0.25 in. column of SE-30 on a mixture of equal parts of Chromosorbs W and G showed that it contained 53% of the diol and 43% of the monoacetates. At 80°C, the diol had a retention time of 1.75 min and the monoacetates a retention time of 2.75 min. The diol could not be separated from the acetates by fractional distillation in the available equipment, but could be separated by extraction. A solution of 1200 g of the mixture in 2 l. of water was extracted four times with 400-ml portions of ether. The combined ether extracts were washed with ten 50-ml portions of water, the ether solution dried over anhydrous magnesium sulfate, and the ether removed by distillation. On examination by GLC, the residue proved to be almost entirely a mixture of the two monoacetates of 3-butene-1,2-diol (181.5 g).

A solution of chromic acid was prepared by mixing 267 g of chromic oxide with 400 ml of water and 230 ml of concentrated sulfuric acid, followed by dilution to 1000 ml. This solution (175 ml) was added over a period of 1 hr to 130 g (1 mol) of the monoacetates dissolved in 1000 ml of acetone, keeping the reaction mixture at 0 to 10°C. The mixture was stirred for another hour at this temperature, diluted with 1000 ml of water, and neutralized with about 90 g of sodium carbonate. The bulk of the acetone was distilled from the reaction mixture and the residue extracted with three 500-ml portions of ether, the extracts dried over anhydrous magnesium sulfate, and the volatile organic materials distilled from the extracts. The residue was further dried by azeotropic distillation with benzene. Fractional distillation resulted in 61 g of material, bp 68–75°C (4 mm). On examination by infrared, NMR, and mass spectroscopy, it proved to be a mixture of 2-keto-3-butenyl acetate and 2-acetoxy-3-butenol.

A total of 104 g of such a mixture was placed in a 1-l. autoclave, and cooled to −60°C, and 66 g of methyl mercaptan added together with 0.6 ml of a 40% solution of Triton B. The reaction mixture was held at 50°C for 3 hr, and then held for 16 hr at room temperature. The excess methyl mercaptan was vented through a sodium hydroxide scrub-

Table III. Results of First Chick Feeding Test[a]

Group	Diet supplement	Av body wt, g			Av feed consumption, g/chick	Av feed conversion, feed/gain
		Initial	Final[b]	Gain		
1	None	36	290 ± 15	254	431	1.70
2	0.2% DL-methionine	38	452 ± 5	414	589	1.42
3	0.5% DL-methionine	37	442 ± 13	405	570	1.41
4	0.2% 4-methylthiobutane-1,2-diol	36	347 ± 16	311	506	1.63
5	0.5% 4-methylthiobutane-1,2-diol	36	420 ± 8	384	562	1.46
6	0.2% 4-thiomethyl-2-butanone	37	265 ± 23	228	415	1.82
7	0.5% 4-thiomethyl-2-butanol	37	256 ± 14	219	450	2.05

[a] Experimental period: 21 days. [b] Mean ± standard error.

Table IV. Results of Second Chick Feeding Test[a]

Group	Diet supplement[b]	Av body wt, g			Av feed consumption, g/chick	Av feed conversion, feed/gain	Survivors[e]
		Initial	Final[c,d]	Gain[c]			
1	None	38	224 ± 12	186	360	1.94	10
2	0.2% DL-methionine	38	401 ± 15	363	551	1.52	10
3	0.2% 4-methylthiobutane-1,2-diol (from MHA ester)	36	264 ± 14	228	407	1.79	10
4	0.2% 4-methylthiobutane-1,2-diol	37	276 ± 11	239	432	1.81	10
5	0.2% 2-amino-4-thiomethylbutanol	39	174 ± 9	136	287	2.11	9
6	0.3% 4-methylthiobutane-1,2-diol diacetate	38	264 ± 20	226	393	1.74	8
7	0.6% 4-methylthiobutane-1,2-diol monolinoleate	38	216 ± 17	176	347	1.97	10
8	1.0% 4-methylthiobutane-1,2-diol dilinoleate	37	265 ± 16	228	422	1.85	9
9	0.2% DL-methionine + 4% corn oil	39	420 ± 17	381	564	1.48	10
10	0.2% 4-methylthiobutane-1,2-diol + 4% corn oil	39	339 ± 17	300	520	1.73	10
11	0.35% 4-methylthiobutane-1,2-diol succinate	38	203	165	303	1.84	4
12	0.2% DL-homocysteine	38	388 ± 20	350	537	1.53	8
13	0.4% DL-homocysteine	38	406 ± 23	368	541	1.47	10
14	0.2% DL-homocystine	38	412 ± 21	374	566	1.51	10
15	0.23% DL-homocysteine thiolactone hydrochloride	36	314 ± 22	278	435	1.56	9
16	0.25% 4-methylthio-2-ketobutyl acetate	38	224 ± 15	186	357	1.92	9

[a] Experimental period: 21 days. [b] Kept isocaloric with control diet by adjusting dietary fiber and carbohydrate. [c] Based on number of surviving chicks. [d] Mean ± standard error. [e] Sacrificed runts and perotic chicks.

bing system and the reaction product distilled. The highest boiling fraction was a solid which on examination by infrared and NMR spectroscopy proved to be the desired 2-keto-4-thiomethylbutyl acetate.

4-Thiomethyl-2-butanone. Methyl vinyl ketone, 109.6 g (1.57 mol), was placed in 1-l. autoclave, the autoclave was cooled with Dry Ice–acetone, and 72 g (1.50 mol) of methyl mercaptan added followed by 1 ml of 40% Triton B (trimethylbenzylammonium hydroxide). The mixture was heated at 50°C for 2.5 hr, then it was distilled under reduced pressure. A fraction boiling at 29–30°C (0.5 mm) weighed 155 g (88% yield) and was shown by gas chromatography to be a single compound. The structure was confirmed to be that of 4-thiomethyl-2-butanone by ir and NMR spectroscopy.

4-Thiomethyl-2-butanol. To 369 ml of a 1.8 *M* solution of methyllithium in ether (0.664 mol) was added at 0°C 62.4 g (0.60 mol) of methylthiopropionaldehyde. After 45 min at 0°C the mixture was heated to reflux for 1 hr and then cooled to 0°C and 66 ml of 10 *M* hydrochloric acid in 100 ml of water was added. The aqueous layer was separated and extracted with ether; then the extracts were combined with the original organic layer and were washed with water until free of acid and dried over $MgSO_4$ and the ether evaporated. The residue, 67.4 g, was distilled. The fraction boiling at 46–47°C (0.6 mm) weighed 38.2 g. The ir and NMR spectra for this fraction were consistent with those expected for 4-thiomethyl-2-butanol.

Rat Feeding Experiments. Crystalline amino acids, in the proportions prescribed by Rogers and Harper (1965),

Table V. Results of Third Chick Feeding Test[a]

Group	Diet supplement[b]	Av body wt, g			Av feed consumption, g/chick	Av feed conversion, feed/gain	Survivors[e]
		Initial	Final[c,d]	Gain[c]			
1	None (3% fat)	40	567 ± 10	527	1126	2.14	10
2	3% HEF (6% fat)	42	547 ± 30	505	1078	2.13	9
3	6% HEF (9% fat)	41	577 ± 28	536	1135	2.12	10
4	0.075% 4-methylthiobutane-1,2-diol	42	516 ± 32	474	1071	2.26	10
5	0.075% 4-methylthiobutane-1,2-diol + 3% HEF	41	646 ± 19	605	1230	2.03	9
6	0.075% 4-methylthiobutane-1,2-diol + 6% HEF	42	610 ± 23	568	1195	2.10	10
7	0.075% methionine hydroxy analog (MHA)	42	627 ± 24	585	1125	1.92	10
8	0.075% MHA + 3% HEF	40	642 ± 14	602	1163	1.93	10
9	0.075% MHA + 6% HEF	41	641 ± 18	600	1194	1.99	10
10	0.075% DL-methionine (DL-Met)	41	668 ± 23	627	1251	2.00	10
11	0.075% DL-Met + 3% HEF	40	670 ± 13	630	1090	1.73	8
12	0.075% DL-Met + 6% HEF	42	624 ± 18	582	1167	2.01	9
13	0.15% 4-methylthiobutane-1,2-diol	41	655 ± 25	614	1156	1.88	10
14	0.15% 4-methylthiobutane-1,2-diol + 3% HEF	40	679 ± 17	639	1183	1.85	10
15	0.15% 4-methylthiobutane-1,2-diol + 6% HEF	39	672 ± 14	633	1260	1.99	10
16	0.15% MHA	40	675 ± 23	635	1150	1.81	9
17	0.15% MHA + 3% HEF	40	663 ± 10	623	1185	1.90	10
18	0.15% MHA + 6% HEF	40	630 ± 25	590	1163	1.97	9
19	0.15% DL-Met	41	685 ± 11	644	1142	1.77	9
20	0.15% DL-Met, 3% HEF	41	677 ± 16	636	1176	1.85	10
21	0.15% DL-Met, 6% HEF	41	645 ± 13	604	1161	1.92	10
22	0.075% 4-methylthiobutane-1,2-diol, 3% lard	40	630 ± 17	590	1172	1.99	10
23	0.15% 4-methylthiobutane-1,2-diol, 3% lard	41	653 ± 19	612	1199	1.96	10
24	0.075% 4-methylthiobutane-1,2-diol, 3% corn oil	40	615 ± 28	575	1167	2.03	10
25	0.15% 4-methylthiobutane-1,2-diol, 3% corn oil	42	687 ± 22	645	1229	1.91	9

[a] Experimental period: 21 days. [b] All diets were kept isocaloric by adjusting the dietary concentration of fiber and carbohydrate (Cerelose). [c] Averages based on surviving chicks. [d] Mean ± standard error. [e] Perotic chicks sacrificed.

were mixed with agar gel. For the diet containing neither methionine nor diol 1, an equal weight of ammonium sulfate was added. The master batch of amino acids was used to prepare rat diets as follows: amino acid mixture, 19%; salt mixture, 5.0%; corn oil, 5.0%; vitamin mixture, 1.0%; corn starch, 35.0%; and sucrose, 35.0%. The amount of methionine in the diet, net of the agar content, was 0.1%. It should be noted that the cages used for the rats did not preclude coprophagia. The results of the feeding tests are summarized in Tables I and II.

Chick Feeding Tests. Ten Vanguard cockerels maintained in batteries were assigned to each diet. Each bird was weighed daily. The basal diet used in the first chick feeding experiment was: 24% soy isolate, 5% mixed minerals, 3% corn oil, 0.2% vitamin mix, 0.3% choline chloride (70%), 3.0% fiber, 14.0% corn starch, 0.0125% Santoquin, and the balance Cerelose. The results of this first test are shown in Table III.

This same diet was also used in the second chick feeding test, the results of which are shown in Table IV. This test gave the first sign that an increase in the fat content of the diet would increase the effectiveness of the diol. This finding was further tested in a third feeding test, the results of which are shown in Table V. For this feeding test, the following basal diet was used: 35% soybean meal (50% protein), 15% corn meal, 2.0% dicalcium phosphate, 1.0% limestone, 0.1% minerals mix, 0.5% salt, 0.2% vitamin mix, 0.1% choline chloride (70%), 0.0125% Santoquin concentrate, 2.6% HEF (Procter and Gamble Company's combination of animal and vegetable fats), and Cerelose to make 100%. More HEF was added to the basal diet of the several groups as shown in Table V. It proved possible to secure enough of the D- and L-4-methylthiobutane-1,2-diol isomers to feed chicks with these materials as supplements to a methionine deficient diet. The results of the experiment are shown in Table VI.

The vitamin mix in all the chick diets had the following composition: ascorbic acid, 12.38%; biotin, 0.03%; vitamin B_{12}, 0.001%; calcium pantothenate, 1.0%; folic acid, 0.20%; Heterogen K, 0.25%; niacin, 7.43%; pyridoxine hydrochloride, 0.30%; riboflavine, 0.8%; thiamine hydrochloride, 1.24%; α-tocopherol acetate (500 IU/g), 1.00%; vitamin A (100000 IU/g), 4.95%, vitamin D_3 (250000 IU/g), 0.12%; sucrose, 70.3%. The mineral mix in all the chick diets had the following composition: calcium carbonate, 5.66%; tricalcium phosphate, 52.80%; dipotassium hydrogen phosphate, 16.97%; magnesium sulfate (anhydrous), 2.36%; ferrous gluconate, 4.22%; zinc chloride, 0.04%; potassium iodide, 0.08%; cupric sulfate (pentahydrate), 0.04%; boric acid, 0.02%; cobaltous sulfate (heptahydrate), 0.002%; manga-

Table VI. Utilization of Optically Active Diol by Chicks[a]

Group	Diet supplement	Av body wt, g: Initial	Av body wt, g: Final[b,c]	Av body wt, g: Gain[c]	Av feed consumption, g/chick	Av feed conversion, feed/gain	Survivors
1	None	36	200 ± 15	164	329	2.01	8[d]
2	0.2% DL-methionine	37	472 ± 10	435	715	1.64	9[e]
3	0.2% DL-4-methylthiobutane-1,2-diol	37	391 ± 35	354	661	1.87	10
4	0.2% D-methylthiobutane-1,2-diol	37	376 ± 32	339	636	1.88	10
5	0.2% L-4-methylthiobutane-1,2-diol	37	403 ± 22	366	699	1.91	9[f]

[a] Experimental period 4 weeks. [b] Mean ± standard error. [c] Averages based on surviving chicks. [d] Two chicks died during second week. [e] One chick died on second day. [f] One chick sacrificed after first week.

nous sulfate (monohydrate), 1.23%; and sodium chloride, 16.59%.

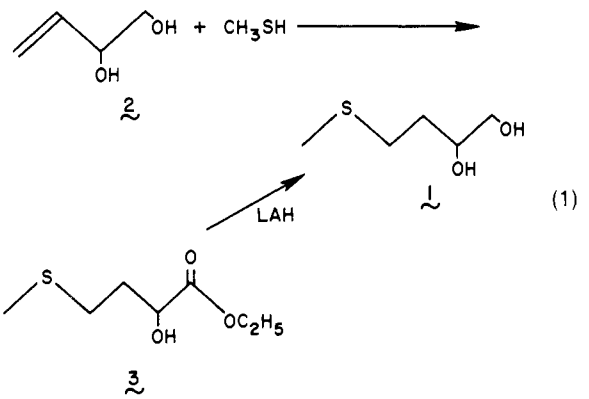
Injection Experiments. One hundred day-old chicks were fed a standard starter mash (Delaware Valley Farmer's Cooperative Association, Flemington, N.J.) for 14 days. At the end of this period, they were starved for 16 hr and then fed for 4 days on the following methionine-deficient diet: 24% soy isolate; 5% mixed minerals; 6% corn oil; 0.2% vitamin mix; 0.3% choline chloride (70%); 14% cornstarch; 0.0125% Santoquin; balance Cerelose. Forty chicks were selected from the flock on the basis of uniformity in body weight and divided into five groups for treatment as shown in Table VII. All groups received the methionine-deficient diet. Group 1 received subcutaneous injections of physiological saline during the entire period at 3-hr intervals except between 1 a.m. and 7 a.m. Group 2 received injections on the same schedule of 0.012 g of DL-methionine dissolved in the least necessary quantity of physiological saline. Group 3 received injections on this schedule of 0.012 g of 4-methylthiobutane-1,2-diol in a similar volume of physiological saline. The diets of groups 4 and 5 were respectively supplemented with 0.2% of DL-methionine and 0.2% of diol 1, and in addition the chicks received injections of physiological saline on the same schedule and at the same fluid dose volume as test groups 1, 2, and 3. The results of the experiment are shown in Table VII.

4-Methylthiobutane-1,2-diol from Chick Droppings. Wet droppings (260 g) from chicks receiving 0.5% of diol 1 in the first feeding experiment (group 5, Table III) were dried at 60°C for 30 hr, yielding 67 g of dry residue. Fifty grams of the dried droppings was extracted with benzene for 24 hr in a Soxhlet extractor. The benzene extract was dried with anhydrous magnesium sulfate and distilled. A nonvolatile residue weighing 2.9 g from this procedure on examination by GLC proved to contain a major component with a retention time identical with that for diol 1. A small quantity of this component was isolated by a preparative GLC technique. Part of the isolated material was used for a mass spectroscopy examination which confirmed the preliminary identification. The remaining material was dissolved in methanol and the optical rotation, $[\alpha]_D$, was found to be +8.5°. This indicates an optical excess of 20.6% of the D-isomer.

DISCUSSION OF RESULTS

Synthesis. 4-Methylthiobutane-1,2-diol was made in high yield in two different ways: (1) addition of methyl mercaptan to 3-butene-1,2-diol (2) and (2) lithium aluminum hydride ($LiAlH_4$) reduction of ethyl 2-hydroxy-4-methylthiobutyrate (methionine hydroxy analog, or MHA), 3 (eq 1).

The desired addition of mercaptan to the starting material 2 was brought about by a free-radical process initiated by a peroxide. The extent of the reaction after initiation



was limited by a chain termination reaction which was not characterized. The problem was overcome for practical purposes by periodic additions of peroxide to the reaction mixture until nearly all the starting materials had been consumed. At temperatures above 75°C, a competing nucleophilic addition of mercaptan to the double bond took place. The products of this reaction, the two diastereomers of 3-methylthiobutane-1,2-diol, were isolated by preparative gas–liquid chromatography (GLC) and identified by mass spectroscopy.

The L-isomer of 1 was obtained easily and in a high state of purity by $LiAlH_4$ reduction of L-2-hydroxy-4-thiomethylbutyric acid. This in turn was obtained by deamination of L-methionine using Akobe's method. Akobe (1936) showed that the hydroxy acid produced in this way has the same optical rotation as the biologically produced L-hydroxy acid. Retention of configuration during the deamination of α-amino acids has been observed also by Brewster et al. (1950). The D-isomer of 1 was obtained by resolution of racemic material. Since diol 1 has no salt-forming functional groups, we introduced acid functionality by forming the bisphthalate half-ester. This was resolved via the L-amphetamine salt. The D-isomer of 1 was obtained in 90% optical purity by acid decomposition of salt which had been recrystallized five times followed by saponification. To further our understanding of the metabolism of compounds structurally related to methionine we also prepared the diacetate of 1, 4-methylthio-2-aminobutanol and 4-methylthio-1-acetoxy-2-butanone.

Rat Feeding Experiments. Compound 1 was first fed to rats receiving a diet of crystalline amino acids described by Rogers and Harper (1965). The rats in the first experiment responded well to the test diet containing the diol 1, as shown in Table I. A second and longer-lasting experiment gave essentially the same results. These two experiments demonstrated clearly that on a weight basis the diol 1 is at least the full equivalent of methionine in the diet of rats. In view of the fact that the molecular weight of the diol 1 is 136, almost 10% less than the molecular weights for methi-

Table VII.[a,b] Tests on Chicks with Injected Supplements

Group	Treatment: Injection	Treatment: Oral supplement	Av body wt, g[c]: Initial	Av body wt, g[c]: 72 hr	Av body wt, g[c]: Gain	Av food intake, g/day per chick: 24 hr: Diet	24 hr: Supplement	48 hr: Diet	48 hr: Supplement	72 hr: Diet	72 hr: Supplement	Total: Diet	Total: Supplement	Av feed conversion, feed/gain
1	Saline	None	272 ± 6	310 ± 7	38	30.4	0	29.5	0	31.5	0	91.4	0	2.40
2	DL-Methionine	None	268 ± 7	335 ± 13	67	35.4	0.084	36.6	0.084	35.8	0.084	107.8	0.252	1.61
3	4-Methylthiobutane-1,2-diol	None	268 ± 6	321 ± 8	53	32.6	0.084	33.1	0.084	34.8	0.084	100.5	0.252	1.90
4	Saline	0.2% DL-methionine	269 ± 7	346 ± 11	77	44.2	0.089	38.4	0.077	41.5	0.083	124.1	0.249	1.61
5	Saline	0.2% 4-methylthiobutane-1,2-diol	265 ± 7	322 ± 10	57	30.9	0.062	37.0	0.074	38.5	0.077	106.4	0.213	1.87

[a] Experimental period: 72 hr. [b] All animals received the same methionine-deficient diet. [c] Mean ± standard error.

onine (149) and MHA (150), we predict that more exhaustive rat feeding experiments would show about a 10% advantage in weight efficiency for compound 1 over methionine and MHA. These results strongly supported the working hypothesis that compound 1 could be metabolized in warm-blooded animals via oxidation of the primary hydroxyl group. This interpretation should be tempered with the knowledge that the coprophagic habits of rats may have permitted them to obtain methionine or methionine hydroxy analog via bacterial action on diol which they might have excreted.

Chick Feeding Experiments. The utility of the diol 1 in chick diets was studied because of its prospective economic importance as a replacement for either methionine or MHA. When the early feeding tests showed the diol to be inferior to methionine (cf. Table III), variations in the makeup of the diet were tried to see if any of the components might influence its metabolism. The most effective way of improving the utilization of the diol was to add fat to the diet (cf. Tables IV and V). This approach was prompted by the work of Baldini and Rosenberg (1959) which showed the synergistic effect of fat on the growth of chicks fed a diet supplemented with MHA. Even with an optimum level of fat in the diet (6%), about 25% more diol than MHA was required for an equivalent growth response. Still, the response to diol was inferior in respect to both weight gain and feed utilization. We are unable to offer a hypothesis to explain how and why a higher level of fat in the chick diet promotes the utilization of the diol.

Analysis of droppings from chicks receiving diol in their diets showed unchanged diol to be present, with a 21% excess of the D-isomer. Quantitative input–output experiments were not made. The excess of D-isomer in the droppings must be interpreted to mean that the L-isomer is more efficiently absorbed from the chick's gut than is the D-isomer. A further indication of this was obtained in a chick feeding test in which the nutritional responses of chicks to 100% L-diol, 71% D-diol–29% DL-diol, and 100% DL-diol were compared. The results (cf. Table VI) do not show clearly differences in the nutritional responses to these supplements owing to the unusually large deviations in the average weight gains. However, the trend of the data does tend to favor the conclusion that the L-diol is more effective than the D-diol. Furthermore, these findings show that incomplete absorption of the diol may be one reason why it is not as effective as methionine.

Subcutaneous injection of diol 1 into selected groups of chicks was done over a period of 3 days to see if the compound performed as well as methionine. The response of the chicks to injected diol 1 still was inferior to methionine given in the same way (Table VII). It was therefore concluded that both absorption and metabolic conversion are factors limiting the utilization of the diol 1 by chicks.

The greater utilization of diol by rats in comparison with chicks can perhaps be ascribed to the coprophagic behavior of rats. This habit of rats may have led to the assimilation of MHA which could be formed by bacterial action on diol contained in droppings. Chicks, of course, are not coprophagic.

The several chick feeding experiments included tests of several compounds structurally related to the diol 1 as follows: 4-methylthiobutane-1,2-diol diacetate, monolinoleate, dilinoleate, and succinate; 4-methylthio-2-ketobutyl acetate; 2-amino-4-methylthiobutanol; 4-thiomethyl-2-butanone; and 4-thiomethyl-2-butanol.

The diesters of diol 1 were about as effective on a mole equivalent as the parent diol, except for the succinate. The last three named of these compounds and the diol succinate seemed to be toxic to the chicks. The poor utility of 4-methylthioketobutyl acetate was somewhat surprising. Tests were also done with diets containing DL-homocystine and DL-homocysteine, recognized replacements for methio-

nine in chick diets. A test feeding of a diet containing the related compound, DL-homocysteine thiolactone hydrochloride, showed that this substance was only about 75% as effective as methionine, DL-homocystine, and DL-homocysteine.

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Metabolism of the Amino Group of Glutamate in Maize Inbred Lines and Their F_1 Hybrid after Infiltration of α-Ketoglutarate and ^{15}N-Labeled Ammonium Sulfate

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Vacuum infiltration of α-ketoglutarate and $(^{15}NH_4)_2SO_4$ was carried out in excised leaves of plants from maize inbred lines and their F_1 hybrid at the sixth true leaf phase. It was established on the basis of the ^{15}N enrichment of the free and protein glutamate, and of the remaining free and protein amino acids, that the processes connected with the operation of the α-ketoglutarate–glutamate system are more intense in the hybrid than in the parent inbred lines. The processes involved are: the reductive amination of α-ketoglutarate, the transamination of glutamate with the remaining amino acids, and the oxidative deamination of glutamate.

The studies described here constitute continuation of our previous work during which differences were established between maize inbred lines and their F_1 heterotic hybrids as regards the changes in the content of some of the free amino acids following infiltration of keto acids and ammonium sulfate in leaf tissue (Mladenova and Istatkov, 1968). A change in the glutamate content, depending on the genotype and on the development phase of the plants, was established under these conditions.

Heavy nitrogen (^{15}N) was used as an indicator in order to obtain indications of the movement of the amino group in processes related to the operation of the α-ketoglutarate–glutamate system after vacuum infiltration of α-ketoglutarate and [^{15}N]ammonium in excised leaves of different maize genotypes.

MATERIALS AND METHODS

Plants used were of the maize inbred lines W-32 and W-187, and their heterotic hybrid W-32 × W-187 cultivated in a greenhouse, at the sixth true leaf phase. The seeds were planted in pots filled with quartzite, and the nutrient solution of Hoagland-Arnon I was used. Vacuum infiltration was carried out by folding 5 g of fresh leaves in gauze packets and by placing them in a beaker containing a 1:1 solution of 0.05 *M* α-ketoglutarate and 0.05 *M* $(^{15}NH_4)_2SO_4$ with a ^{15}N abundance of 12.7 atom %. The beaker was placed in a vacuum dessicator, and vacuum treatment was continued until no more air bubbles appeared. Then the vacuum was gradually released. After infiltration, the samples were washed in distilled water and placed at room temperature in a humid glass chamber. They were inactivated at 105° for 5 min at intervals of 15 and 60 min and then dried to a constant weight; 300 mg was taken from the dried and ground material in order to prepare the amino acid extracts. For the free amino acids, the samples were placed in 80% ethanol, followed by a water-bath extraction. After decantation, the alcohol was evaporated and the dry residue was dissolved in 1 ml of water. After filtration, the filtrate and the rinsing water were passed through cation-exchange resin KPS (in H^+ form).

Electrophoretic separation was carried out with purified amino acid solutions for 40 to 50 min at 500 V, using 0.1 *M* pyridine–acetic acid buffer (pH 5.4). A ninhydrin solution was used for the visualization of the amino acid bands on the electropherograms. After extraction of the free amino acids, the protein amino acids were obtained after hydrolysis of 100 mg of the ground residue previously dried at 65°. Hydrolysis was carried out with 6 *N* HCl for 48 hr at 110°. The free and protein amino acids separated under the influence of the electric field were grouped in the following

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