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A Convenient Synthesis of DL-*homo*Methionine (5-Methylthio-*nor*valine)

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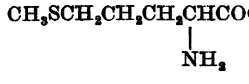
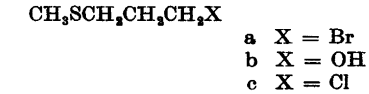
A three-step synthesis of *homo*methionine (I) in an over-all yield of 70 % is described. It proceeds from dimethyl formamidomalonate which is smoothly allylated to (X). UV- and peroxide-catalyzed addition of methyl mercaptan affords in quantitative yield the ester (XI), which is hydrolyzed by acid to give *homo*methionine. The latter is characterized as its N-formyl- and N-benzoyl-derivatives. The phenylthiohydantoin (IX) is prepared upon reaction of (I) with phenyl *iso*thiocyanate.

A similar sequence of reactions is described with the usual, but less conveniently prepared, *diethyl* formamidomalonate as a starting material. Alkylation experiments have demonstrated methylthiopropyl halides to be of little use in the present case.

In the course of studies on the phytochemistry of naturally occurring *iso*thiocyanates a need arose for an authentic specimen of DL-*homo*methionine (5-methylthio*nor*valine) (I).



I



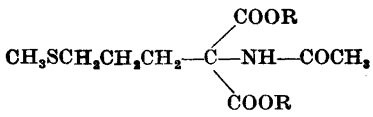
II

This amino acid, which has not been encountered in Nature, was first synthesized in the laboratory of du Vigneaud [1] by a fairly circumstantial procedure, not specifically designed for the synthesis of *homo*methionine. Later Gaudry *et al.*[2] reported rather low yields in the synthesis of (I) from γ-bromopropylhydantoin and methyl mercaptan, followed by hydrolysis with alkali.

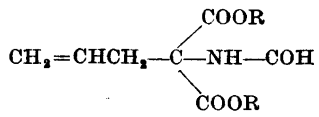
We preferred to build up (I) by alkylation of a suitable α-acylamidoacid ester possessing a reactive α-hydrogen atom. Our first efforts, however, were directed towards the synthesis of the previously unknown 3-methylthiopropyl bromide (IIa) by the addition of methyl mercaptan to allyl bromide. Although reaction took place it was unfortunately accompanied by undesirable side reactions, and this observation led us to reinvestigate the addition of methyl mercaptan to allyl alcohol previously reported by Kaneko [3]. With slight modi-

fications the addition reaction gave *quantitative* yields of (IIb), thus exceeding those of formally analogous reactions, *e. g.* the addition of benzyl mercaptan to allyl alcohol, reported by Brown, Jones and Pinder [4] to proceed in 51—70 % yield, dependent on the type of catalyst. The exchange of the hydroxy-grouping of (IIb) with chlorine was effected with thionyl chloride as described by Kirner [5,6], resulting in a 67% yield of (IIc). An alternative and equally satisfactory way of preparing the latter was found in the preferential reaction at the 1-bromo position of the commercially available 1-bromo-3-chloropropane with sodium methylmercaptide. Further reaction products obtained from the differently prepared chlorides proved identical, a fact which served to confirm that the addition had taken place as desired (contrary to Markownikoff's rule).

Several attempts to alkylate sodium ethyl acetamidocyanoacetate with (IIc) gave crystalline (III) in a maximum yield of only 11 %, large amounts of an intractable brown oil being simultanously formed. Cautious alkaline hydrolysis of (III) afforded the corresponding free acid (IV).



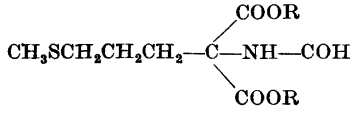
III R = C_2H_5
IV R = H



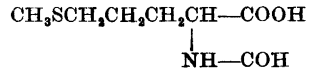
V R = C_2H_5
X R = CH_3

No better results attended numerous attempts in which (IIc) or (IIa) were condensed with sodium ethyl formamidomalonate [7] in stead of the cyanoacetic acid derivative. Again, considerable amounts of dark-coloured, secondary products appeared, possibly due to the formation of sulphonium-type by-products. The unsatisfactory results were rather unexpected in view of the reported smooth condensation of the lower homologous 2-methylthioethyl chloride with sodium ethyl formamidomalonate, an essential step in two methionine syntheses [8,9]. Consequently, further attempts to utilize methylthiopropyl halides were abandoned and other approaches considered.

Ethyl allylformamidomalonate (V) was readily obtained by allylation of sodium ethyl formamidomalonate in almost quantitative yield as described by Koštíř and Král [10]. Addition of methyl mercaptan to (V) under the usual conditions (UV-light, benzoyl peroxide and mercuric acetate) afforded a quantitative yield of the crystalline adduct (VI).



VI R = C_2H_5
XI R = CH_3

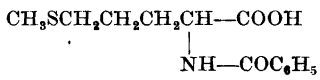


VII

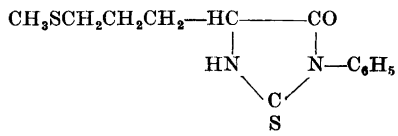
Upon treatment of the latter with the calculated amount of methanolic potassium hydroxide at reflux temperature N-formyl*homo*methionine (VII) resulted; no special effort was made to isolate formamidomethylthiopropyl-

malonic acid which is presumed to be formed as an unstable intermediate in the reaction. The formyl derivative (m. p. 122-123°) was previously used [1] for the characterization of *homo*methionine; our pure preparation was found to melt at 128-129° with some previous sintering.

Acid hydrolysis of either (VI) or (VII) yielded *homo*methionine hydrochloride which was in turn transformed into the free amino acid (I) by passing through an anion exchange resin (Amberlite IR-4B) in its hydroxyl form. The high melting point reported [1] (272-274°) (corr.)) could not be reproduced for our specimen, which heated in capillary tubes melted at *ca.* 235°, whereas rapid heating on the Fisher-John's block gave the value 247-248°, both accompanied by extensive decomposition (Ref.[2] lists m. p. 254-255° (dec.)). For further characterization *homo*methionine was transformed into its N-benzoyl derivative (VIII) and phenylthiohydantoin (IX), the latter prepared by the general method of Edman [11].



VIII



IX

While the present work was in progress a paper by Hellmann and Lingens [12] appeared in which the advantage of using *methyl* formamidomalonate instead of the usual ethyl ester in amino acid syntheses was pointed out. We could confirm the convenient synthesis of the methyl ester in almost quantitative yield as stated, and consequently carried through the above sequence of reactions also in the methyl series. The allylation, addition and hydrolysis all proceeded in nearly quantitative yields. Therefore, this route to *homo*methionine from easily accessible starting materials in an over-all yield of *ca.* 70 % represents a facile and convenient synthesis. With obvious alterations the same sequence of reactions can be utilized for the preparation of a series of analogous S-alkyl homologues of α-amino acids, *e. g. homo*ethionine.

The paperchromatographic behaviour of *homo*methionine was studied in two solvent systems. The R_F-values, together with those of some natural α-amino acids, partly appearing in the same range, are listed in Table 1.

EXPERIMENTAL

All melting points are uncorrected and determined in capillary tubes unless otherwise stated. Those below 80° were determined in a water bath, the remaining ones in an electrically heated block.

3-Methylthiopropanol (IIb). Since Kaneko's work was accessible only in abstract form [3] with few experimental details we were obliged to reinvestigate the optimal conditions for the addition reaction. In a round-bottomed quartz flask were placed 35 ml (0.52 mole) of freshly distilled allyl alcohol together with 100 mg of benzoyl peroxide and 200 mg of mercuric acetate. The solution was cooled and an ampoule containing 15.6 g (0.33 mole) of methyl mercaptan introduced and crushed beneath the surface by means of a glass rod. The flask was tightly stoppered and placed in front of a quartz-mercury lamp for

Table 1. R_F-*Values of* homo*methionine and various other amino acids determined on Whatman paper No. 1 by the descending technique.*

Amino acid	*n*-Butanol/Acetic acid/Water 4:1:3	Pyridine/Amyl alcohol/Water 35:35:30
Glycine	0.15	0.03
Valine	0.47	0.14
Leucine	0.63	0.24
*iso*Leucine	0.61	0.23
Methionine	0.49	0.17
*homo*Methionine	0.54	0.22
Phenylalanine	0.57	0.25
Tyrosine	0.43	0.20

12—16 hours while being cooled by a stream of cold water. After filtration, distillation *in vacuo* afforded 33.2 g (94 %, calc. on methyl mercaptan) of pure 3-methylthiopropanol, b. p. 89—90° at 13 mm. Ref.[5] reports b. p. 93—94° at 17 mm.

3-Methylthiopropyl chloride (IIc). By the modified method[6] of Kirner[5] the above alcohol (84.4 g) was transformed into the corresponding chloride (66.6 g), *i. e.* 67 % yield. B. p. 73.5—74.5° at 28 mm. Reported[5]: b. p. 71.2° at 29 mm.

The same chloride was obtained from 1-bromo-3-chloropropane in the following way. To a well-cooled solution of 3.80 g (0.165 mole) of sodium in 80 ml of anhydrous ethanol was added 7.83 g (0.163 mole) of methyl mercaptan; the dihalide (25.8 g; 0.154 mole) was then introduced, resulting in an immediate reaction. The calculated amount of sodium bromide was removed by filtration and the filtrate fractionated *in vacuo* yielding 16.3 g (85 %) of 3-methylthiopropyl chloride, b. p. 63—64° at 19 mm.

N-Acetyl-2-cyano-2-(3'-methylthiopropyl)-glycine ethyl ester (III). To a solution of 2.30 g (0.10 mole) of sodium in 50 ml of thoroughly dried ethanol were added 17.02 g (0.10 mole) of ethyl acetamidocyanoacetate (commercially obtained from the Sterling-Winthrop Co.), followed by 13.00 g (0.104 mole) of 3-methylthiopropyl chloride. The mixture was refluxed for 20 hours, protected from atmospheric moisture by a calcium chloride tube. The dark-brown mixture was then poured on to 250 g of crushed ice when a brown oil separated, together with smaller amounts of a crystalline product. The latter was isolated by filtration, washed with ice-water and dried, 2.80 g (11 %). After recrystallization from dilute ethanol (Norit) and then twice from ethyl acetate and petroleum ether, colourless needles were obtained, m. p. 80.5—81.5°. (Found: C 51.24; H 6.87; N 10.70; S 12.28. Calc. for $C_{11}H_{18}O_3N_2S$: C 51.15; H 7.02; N 10.85; S 12.42). Experiments conducted with stirring, shorter reaction times, and with the exclusion of air proceeded essentially as described with no improvements of the yield.

N-Acetyl-2-cyano-(3'-methylthiopropyl)-glycine (IV). A solution of 0.79 g (3.05 mmoles) of the above ester in 3.45 ml of 0.89 *N* methanolic KOH and 4 ml of water was left at room temperature for 72 hours. The methanol was removed *in vacuo* and the aqueous solution freed of unreacted ester by extraction with chloroform. The well-cooled aqueous solution was acidified to pH 2 and the colourless precipitate (0.65 g, 93 %) collected by filtration. The product was rapidly recrystallized from water, care being taken not to raise the temperature above 50° where vigorous decarboxylation sets in. The dense, shiny crystals melted with decomposition at 130—131°. (Found: C 47.17; H 6.26; N 11.94; S 14.02. Calc. for $C_9H_{14}O_3N_2S$: C 46.93; H 6.13; N 12.17; S 13.92).

Diethyl allylformamidomalonate (V). This substance was prepared in 96 % yield essentially as described[10]. M. p. 64—65°; Ref.[10] reports m. p. 64°.

Diethyl (3-methylthiopropyl)-formamidomalonate (VI). A cooled solution of 14.0 g (0.057 mole) of the allyl ester (V) in 35 ml of absolute ethanol, containing 30 mg of benzoyl peroxide and 150 mg of mercuric acetate was placed in a quartz flask. 2.95 g (0.061 mole) of methyl mercaptan were added and the cooled and tightly stoppered reaction vessel

was exposed to ultraviolet light for 18 hours. When the reaction was over the contents were brought into solution by moderate heating on the steam bath and a slight, insoluble residue removed by filtration. The filtrate was concentrated *in vacuo* to a colourless oil, care being taken to keep the bath temperature below 45°. The oil was treated with ice-water when it crystallized to give 15.13 g (91 %) of (VI), sufficiently pure for further use. An analytical sample was obtained as thin, shiny plates after three recrystallizations from aqueous ethanol, m. p. 64°, the same as that of the starting material. A mixture, however, melted at 48—50°. (Found: C 49.32; H 7.11; N 4.98; S 11.24. Calc. for $C_{13}H_{21}O_5NS$: C 49.46; H 7.27; N 4.81; S 11.00).

Dimethyl formamidomalonate. This ester was prepared as described [11]. In order to obtain the best yields (*ca.* 95 %) we found it necessary to employ zinc powder activated by short exposure to hydrochloric acid.

Dimethyl allylformamidomalonate (X). Dimethyl formamidomalonate (56.0 g) was alkylated with allyl bromide in dry methanol solution, following the procedure used for the preparation of the ethyl ester. In a preliminary experiment where ethanol was used as a solvent transesterification to the ethyl ester occurred. The yield was 55.0 g (80 %) of crystalline ester, considerably more soluble in water than the ethyl ester above. An analytical specimen was prepared by two recrystallizations from water, m. p. 75—76°. (Found: C 50.30; H 5.97; N 6.65. Calc. for $C_9H_{13}O_5N$: C 50.23; H 6.09; N 6.51).

Dimethyl (3-methylthiopropyl)-formamidomalonate (XI). The addition of methyl mercaptan to (X) was performed as described above in the ethyl series, but with methanol as the solvent. From 19.6 g (0.091 mole) of (X) and 7.20 g (0.15 mole) of methyl mercaptan, 22.9 g (96 %) of almost pure (XI) was obtained. A sample for analysis was recrystallized twice from water, m. p. 72.5°. (Found: C 45.45; H 6.32; N 5.34. Calc. for $C_{10}H_{17}O_5NS$: C 45.60; H 6.51; N 5.32).

N-Formyl-DL-homo*methionine* (VII). The esters, (VI) or (XI), were refluxed for 30 minutes with exactly two equivalents of 0.89 *N* methanolic KOH. Water was added to dissolve the sodium salt and the methanol removed *in vacuo*. The cooled solution was acidified to pH 2 with 6 *N* HCl, accompanied by a vigorous evolution of CO_2. The clear solution was left at 0° overnight when a 55 % yield of (VII) separated. An additional amount was obtained after evaporation of the filtrate to dryness and repeated extractions of the residue with hot ethyl acetate. An analytical specimen was isolated as small rhombic plates from ethyl acetate. M. p. 128—129° after slight sintering from *ca.* 122°. Ref.[1] reports the m. p. 122—123°. (Found: C 43.87; H 6.78; N 7.39. Calc. for $C_7H_{13}O_3NS$: C 43.97; H 6.85; N 7.33).

DL-homo*Methionine* (I). The formyl derivative (VII) or the esters, (VI) or (XI), were hydrolyzed to the amino acid (I). Generally, the compound in question was refluxed with a large excess of constant boiling HCl for several hours. In one experiment, in which HBr was used, a concomitant cleavage of the C—S bond appeared to occur as indicated by the evolution of methyl mercaptan. Excess hydrochloric acid was removed by repeated evaporations with freshly added portions of water. The dilute solution of *homo*methionine hydrochloride was slowly passed through a column of Amberlite IR-4B in its hydroxyl-form (*ca.* 20 g of resin per 5 g of amino acid). The chloride-free eluate was concentrated to dryness *in vacuo*. The residue was dissolved in a small amount of hot water; cautious addition of ethanol resulted in the separation of *homo*methionine in beautiful hexagonal plates. After being kept for 24 hours at 0° the crystals were collected by filtration and dried over P_2O_5 at 100° *in vacuo*. The yield was 85—90 %. The amino acid decomposed at *ca.* 235° in capillary tubes. Upon rapid heating on the Fisher-John's block the m. p. 247—248° (dec.) was observed. (Lit. values: 272—274° (corr.) [1], 254—255° (dec.) [2]). (Found: C 44.23; H 7.95; N 8.75; S 19.50. Calc. for $C_6H_{13}O_2NS$: C 44.16; H 8.03; N 8.58; S 19.64).

N-Benzoyl-DL-homo*methionine* (VIII). To a cooled and stirred solution of 163.2 mg of (I) in 0.85 ml of 1.20 *N* NaOH and 5 ml of water, were simultaneously added 0.14 ml of freshly distilled benzoyl chloride and 0.90 ml of 1.20 *N* NaOH. After stirring at room temperature for an hour the solution was acidified to pH 1—2; the resulting precipitate was filtered off and thoroughly washed with petroleum ether. In this way the benzoyl derivative (245.8 mg) was obtained in 92 % yield. It was recrystallized twice from aqueous ethanol for analysis as colourless needles, m p. 135° (Fisher-John's block). (Found: C 58.38; H 6.18; N 5.10. Calc. for $C_{13}H_{17}O_3NS$: C 58.40; H 6.41; N 5.24).

3-Phenyl-5-(3'-methylthiopropyl)-2-thiohydantoin (IX). A solution of 450 mg of *homo*methionine in 20 ml of 60 % pyridine was brought to pH 8.6 by adding 1 *N* NaOH (0.12 ml). 1.5 ml of phenyl *iso*thiocyanate were added and the solution kept between pH 8.4 – 8.6 by gradual addition of 1 *N* NaOH (2.72 ml used; calc. 2.76 ml). After 2 hours the reaction was over and excess reagent was removed by extraction with benzene. The aqueous phase was covered with 20 ml of ethyl acetate and acidified to pH 2 with 4 *N* HCl. The organic phase was separated, dried and taken to dryness, leaving an oil which was dissolved in 5 ml of glacial acetic acid saturated with dry HCl. The solution was left overnight and taken to dryness at 30 – 40° *in vacuo*, when 670 mg (87 %) of the crystalline thiohydantoin resulted. Two recrystallizations from ethanol afforded an analytical sample with m. p. 111°. (Found: C 55.56; H 5.65; N 10.00; S 22.98. Calc. for $C_{13}H_{16}ON_2S_2$: C 55.68; H 5.75; N 9.99; S 22.87).

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