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On the Preparation of DL-3-Selena- and DL-9-Selena--tuberculostearic Acids

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The syntheses of two selenium-containing branched-chain fatty acids are reported. Through a multistep process a series of higher branched-chain intermediates, as well as some of their derivatives have been prepared. The effect of the incorporation of the selenium atom in two different positions in the chain and the comparison of selenium-containing acids and their amides with their normal analogues, are briefly described.

Previous interest in the biological aspects of selenium has emphasized its toxicity. Now the interest has shifted, largely to its role as a possible trace element. It has been found that it will prevent and control several distinct disease entities occurring in all classes of livestock.[1,2] Since the announcement of the identification of selenium as an integral component of "Factor 3" in 1957 [3] it has become evident that this factor protects against a large variety of deficiency syndromes in numerous species.[4] Although the exact chemical structure of "Factor 3" has not been determined yet, a number of organoselenium compounds with good biopotency, more effective than inorganic selenite, have been prepared.

In the course of current investigations on organoselenium compounds a number of long-chain selena-carboxylic acids have been prepared at this institute. Most of the acids have rather good "Factor 3" activity, but the effect varies with the position of the selenium atom in the chain.[5] As a continuation and extension of the work it was decided to prepare some of the branched-chain selena-fatty acids, and study the effect of the selenium atom incorporated in the different positions in the chain. Thus the preparation of DL-3-selena- and DL-9-selena-tuberculostearic acid was undertaken.

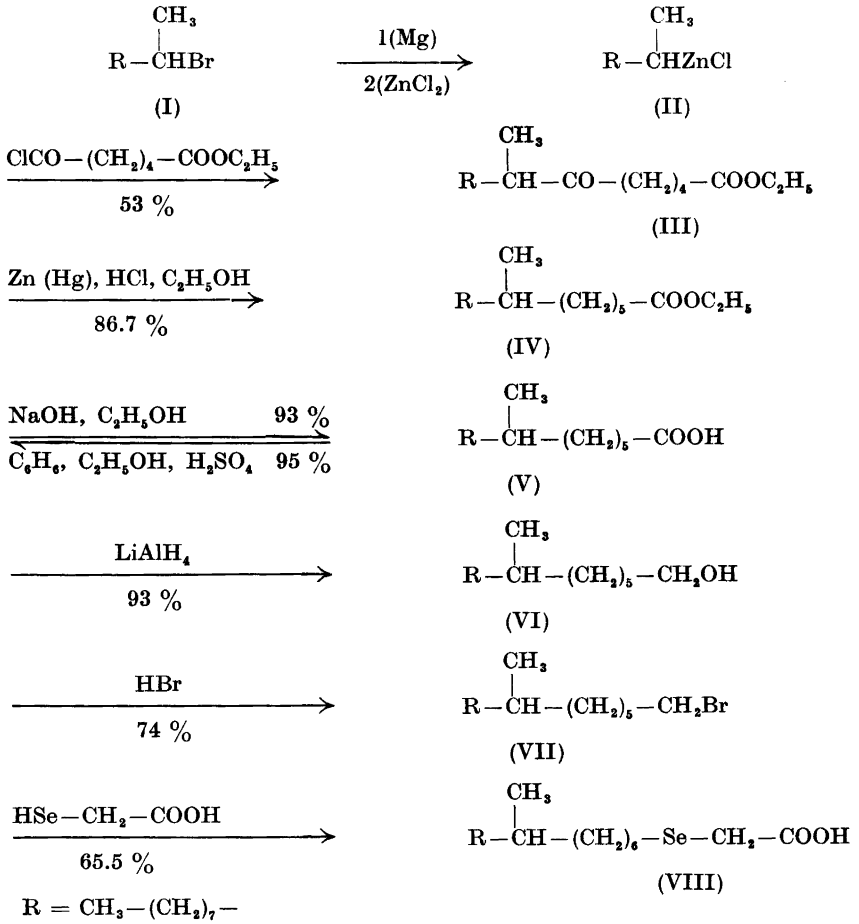
Tuberculostearic acid or (—)-10-methylstearic acid is of historic interest and its racemic and optically active forms have received extensive attention in the literature. It was first isolated from the lipids of tubercle bacillus;[6] later from bovine tubercle wax [7] and leprosy bacillus.[8] Its chemical structure was subsequently assigned [9] and confirmed.[10] During one decade eight syntheses of tuberculostearic acid appeared in the literature.[11–18]

The complete identification of synthetic (−)-10-methyloctadecanoic acid with the acid isolated from the lipids established the structure of tuberculostearic acid to be, beyond doubt, the levorotatory form of 10-methyloctadecanoic acid but with very small optical activity.

As previously stated this work will deal with the preparation of two of the analogous acids containing selenium in the chain, namely DL-3-selena- and DL-9-selena-10-methyloctadecanoic acids.

A. 3-SELENA-10-METHYLOCTADECANOIC ACID

This acid was prepared by the addition of 1-bromo-7-methylpentadecane to an ammoniacal solution of reduced di-seleno-di-acetic acid. 1-Bromo-7-methylpentadecane was synthesized by a multistep preparation including a series of higher branched-chain intermediates, as well as some of their derivatives, which should be of interest in the field of fatty acid chemistry.



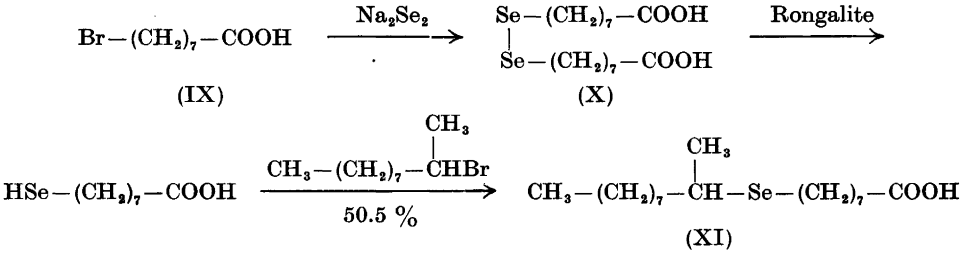
2-Decylzinc chloride [15] (II) was prepared from 2-bromodecane (I) by a Grignard reaction with the subsequent addition of zinc chloride to the solution. The 2-decylzinc chloride reacted with 5-carbethoxyvaleroyl chloride to give ethyl 6-keto-7-methylpentadecanoate (III). This keto ester was reduced by the Clemmensen method and gave ethyl 7-methylpentadecanoate (IV) which was hydrolyzed to its corresponding acid (V). The acid was reduced by $LiAlH_4$ to 7-methylpentadecanol (VI) and the alcohol was converted to 1-bromo-7-methylpentadecane (VII). This halide, as already mentioned, was allowed to react with an ammoniacal solution of selenol acetic acid. Acidification of the solution gave impure 3-selena-10-methyloctadecanoic acid (VIII). The acid was an oily liquid and purification was not possible either by distillation, which caused some decomposition, or by chromatography (alumina column). It was converted to its more easily handled S-(*p*-chlorobenzyl)-thiuronium salt and after purification of this derivative the acid was isolated in pure form. 3-Selena-10-methyloctadecanamide was also conveniently prepared from the acid in a mixed solution of oxalyl chloride and dry benzene and the subsequent addition of cold concentrated ammonia to the acid chloride after removal of the excess oxalyl chloride and benzene.

The syntheses and data on yields are given in the scheme above.

B. 9-SELENA-10-METHYLOCTADECANOIC ACID

ω-Bromocaprylic acid (IX) was prepared from ethyl hydrogen azelate according to the Hunsdiecker and Hunsdiecker method. The halo acid was converted to ω,ω'-di-seleno-dicaprylic acid (X). The diselenide was reduced in ammoniacal solution by Rongalite and 2-bromodecane was added to the solution. 9-Selena-10-methyloctadecanoic acid (XI) was liberated from the alkaline solution by acidification and its amide was also prepared.

The synthesis is shown schematically below.



A comparison of some of the physical constants of DL-10-methyloctadecanoic acid and the analogous selenium-containing acids and their amide derivatives is shown in Table 1.

EXPERIMENTAL

A. 3-Selena-10-methyloctadecanoic acid

2-Decanol. This alcohol was prepared by the reaction of octylmagnesium bromide and acetaldehyde in the customary manner yielding 82 %; b.p. 108—110° (18 mm), n_D^{20} 1.4338.

Table 1.

Acid	Refractive index		m.p. of acid	m.p. of amide
DL-10-Methyloctadecanoic (Prout *et al.*[13])	n_D^{25}	1.4512	25.4—26.1°	77.5—79.2°
DL-3-Selena-10-methyloctadecanoic	n_D^{20}	1.4864	liquid	45—47°
DL-9-Selena-10-methyloctadecanoic	n_D^{20}	1.4855	16—17.5°	78.5—80.5°

2-Bromodecane (I). 2-Decanol was converted to 2-bromodecane according to the procedures of Hsueh and Marvel [19] for the preparation of DL-2-bromooctane, giving a yield of 85 %; b.p. 108° (13 mm), n_D^{20} 1.4540.

Ethyl hydrogen adipate. Diethyl adipate was prepared according to the procedure of Micovic [20] yielding 94 %. The ester and adipic acid were used for the preparation of the half ester in accordance with the procedure described by Swann *et al.*[21] for the preparation of hydrogen sebacate. The half ester was obtained in 70 % yield on a one-mole scale; b.p. 160—162° (10 mm).

5-Carbethoxyvaleroyl chloride. A mixture of 162 g ethyl hydrogen adipate and 145 g thionyl chloride was allowed to stand at room temperature over-night. The excess thionyl chloride was removed and the 5-carbethoxyvaleroyl chloride distilled in vacuum. It boiled at 128° (17 mm) and the yield was 172 g (96 %).

Ethyl 6-keto-7-methylpentadecanoate (III). This keto ester was prepared following the method used by Jones [22] in the preparation of the long-chain keto acids and the procedure of Schmidt and Shirley [15] using a secondary organozinc compound in the synthesis of the branched long-chain keto acid: A solution of 222.1 g (1 mole) 2-bromodecane in 500 ml anhydrous ether was added in the usual manner to 39 g magnesium turnings and a small iodine crystal in 100 ml absolute ether. The yield of this reaction through titration of the resulting Grignard solution has been shown to be 80 %.[15] 109 g (0.8 mole) freshly fused anhydrous zinc chloride and 250 ml dry ether were placed in a 2-litre, three-necked flask provided with a stirrer together with a reflux condenser and a dropping funnel fitted with calcium chloride tubes. The ethereal 2-decylmagnesium bromide solution was added from the dropping funnel at such a rate that gentle reflux was maintained. This mixture was refluxed gently for 2 h during which time the ether was allowed to distil slowly until the volume had been reduced to 400 ml. A solution of 115.6 g (0.6 mole) 5-carbethoxyvaleroyl chloride in 350 ml of dry benzene was added to the resulting mixture with continued stirring. This mixture was refluxed for three hours and hydrolyzed with excess water containing a little hydrochloric acid. The organic layer was separated and dried over magnesium sulphate. The volatile organic material was removed by distillation until a head temperature of 140° (10 mm) was reached. The residue was treated with ethanol under esterification conditions using benzene and a few ml of sulphuric acid to esterify ethyl hydrogen adipate (from unreacted acid chloride). In this way the efficiency of the separation of the more volatile diethyl ester by distillation from the keto ester is increased. Diethyl ester was distilled off through a Vigreux column and the rest was distilled twice through a 82 cm × 1.2 cm column, packed with 1/4 inch glass Raschig rings and wrapped with an electrically heated jacket. Ethyl 6-keto-7-methylpentadecanoate weighed 95 g; yield 53 % based on the starting amount of 5-carbethoxyvaleroyl chloride. It boiled at 160—162° (0.5 mm), n_D^{20} 1.4530. (Found: C 72.30; H 11.20. Calc. for $C_{18}H_{34}O_3$: C 72.43; H 11.48).

6-Keto-7-methylpentadecanoic acid. 12 g of ethyl 6-keto-7-methylpentadecanoate were added to a solution of 25 g sodium hydroxide dissolved in 100 ml ethanol and 50 ml water and refluxed for four hours. The alcohol was distilled off and the reaction mixture was diluted with water and acidified. The oily product was extracted with ether and dried over magnesium sulphate. The ether was removed and the keto acid was distilled at 171—172° (0.8 mm) and solidified in the refrigerator. It weighed 9.5 g; yield 87 %. A small

amount of the keto acid was recrystallized twice from acetone and from petroleum ether at −20° and gave a pure product, m.p. 35−36.5°. (Found: equiv. wt. 269.5. Calc. for $C_{16}H_{30}O_3$: equiv. wt. 270.4).

A semicarbazone derivative of the keto acid was prepared with 82 % yield, according to Shriner and Fuson.[23] It was recrystallized three times from acetone and melted at 112−114°. (Found: N 12.98. Calc. for $C_{17}H_{33}N_3$: N 12.84).

Ethyl 7-methylpentadecanoate (IV). Following the general method used by Schneider and Spielman [24] for a Clemmensen reduction, 75 g ethyl 6-keto-7-methylpentadecanoate were added to 1.5 l absolute ethanol. Then 700 g amalgamated zinc [22] were added to the solution, which was saturated with dry hydrogen chloride and refluxed with stirring for 24 h. It was saturated again with hydrogen chloride and refluxed for an additional 36 h. The unreacted zinc was removed and the solution was reduced to one-half of its volume by distillation. Water was added and the organic layer was extracted twice with benzene and dried over magnesium sulphate. The benzene was removed and the residue distilled through the long column, described in connection with the distillation of keto ester. It boiled at 138−140° (0.9 mm) and weighed 62 g; yield 86.7 %, n_D^{20} 1.4411. The ester was impure even after distilling it many times; however, the pure ester was obtained from pure acid (the preparation of which is described below). Yield 95 %; b.p. 131−132° (0.8 mm), n_D^{20} 1.4407. (Found: C 76.40; H 12.96. Calc. for $C_{18}H_{36}O_2$; C 75.99; H 12.76).

7-Methylpentadecanoic acid (V). 56 g ethyl 7-methylpentadecanoate were added to a mixture of 300 ml ethanol and 100 ml 40 % aqueous sodium hydroxide. It was refluxed for 15 h and acidified with 2 N hydrochloric acid. The acid layer was extracted by means of ether and was washed several times with water and dried over magnesium sulphate. The ether was removed and the remaining acid distilled at 153−155° (0.9 mm) giving 47 g, yield 93.1 %, which solidified in the condenser; m.p. 23−26°. A small amount of the acid was recrystallized three times from acetone by cooling the acetone solution in an ice-concentrated hydrochloric acid mixture. The acid melted at 25.5−27°. (Found: equiv. wt. 257. Calc. for $C_{16}H_{32}O_2$: equiv. wt. 256.4).

7-Methylpentadecanamide. 2 g 7-methylpentadecanoic acid and 6 ml thionyl chloride were refluxed for an hour. The excess thionyl chloride was removed and 40 ml cold concentrated ammonia were added to the acid chloride and shaken for 5 min. The crystalline crude amide melted at 67−70° and weighed 1.6 g. Yield 80 %. It was recrystallized once from methanol and twice from acetone giving a pure product which melted at 70−70.5°. (Found: N 5.55. Calc. for $C_{16}H_{33}NO$: N 5.49).

The 2,4,6-tribromoanilide of the acid was prepared according to Cason [25] by preparing the acid chloride from 1 g of acid and heating it with 1.2 g of tribromoaniline for 2 h on a steam-bath. After three recrystallizations from methanol it melted at 96.5−97°; yield 72 %. (Found: C 46.76; H 5.91. Calc. for $C_{22}H_{34}Br_3NO$: C 46.50; H 6.03).

7-Methylpentadecanol (VI). The crude acid (m.p. 23−26°) was reduced by $LiAlH_4$ in the usual way. 400 ml of dried ether were placed in a 1 l three-necked flask equipped with a stirrer, reflux condenser, and a dropping funnel. 4 g of $LiAlH_4$ were added to the solution and carefully stirred. 25 g 7-methylpentadecanoic acid were dissolved in 200 ml of dry ether and added to the ethereal solution from the dropping funnel at such a rate that a gentle reflux was maintained. After addition of the acid the ether solution was refluxed for half an hour. The mixture was cooled and 150 ml of ether saturated with water were added very slowly and carefully to the solution. Thereafter 18 ml of 2 N sulphuric acid were added very slowly. The solution was filtered through a glass filter and dried over magnesium sulphate. The ether was removed and the alcohol distilled at 134−136° (1 mm); n_D^{20} 1.4507. The alcohol weighed 22 g; yield 93 %. (Found: C 79.30; H 14.09. Calc. for $C_{16}H_{34}O$: C 79.26; H 14.14).

1-Bromo-7-methylpentadecane (VII). 7-Methylpentadecanol was treated with dry hydrogen bromide using the procedure of Reid, Ruhoff and Burnett.[26] 20 g alcohol were heated to 100° and dry hydrogen bromide was passed into the liquid while the temperature was maintained between 100 and 120° at such a rate that saturation was attained after 1.5 h. The crude bromide was separated from the aqueous hydrobromic acid formed during the reaction and was shaken with 6 ml concentrated sulphuric acid. The bromide layer was separated and mixed with an equal volume of 50 % methyl alcohol. During shaking some emulsion appeared. An ammonium hydroxide solution was added to the mixture until the solution became alkaline to phenolphthalein. The complete separation of the solution took place after 2 h. The bromide layer was washed

again with 15 ml of 50 % methyl alcohol. It was dried over magnesium sulphate and distilled at 148—150° (2 mm). n_D^{20} 1.4628. The bromide weighed 18.7 g; yield 74 %. (Found: Br 26.16. Calc. for $C_{16}H_{33}Br$: Br 26.17).

Diselenodiglycolic acid. This acid was prepared by the reaction of potassium selenocyanate [27] with potassium chloroacetate to give its selenocyanoacetate which in turn was converted to diselenodiglycolic acid.[28,29]

3-Selena-10-methyloctadecanoic acid (VIII). 4.5 g diselenodiglycolic acid were dissolved in 75 ml 2 N ammonium hydroxide solution in a closed flask. To this excess Rongalite was added until the yellow colour of the solution disappeared. 10 g 1-bromo-7-methylpentadecane in 100 ml ethanol was added to the solution and it was shaken for 12 h. As the colour of the solution became yellow more Rongalite was added and it was shaken for another 12 h. The solution was diluted with water to four times its volume and acidified with 2 N hydrochloric acid. A reddish yellow colour and an oily product appeared. The oily material was extracted with ether and dried over magnesium sulphate. The ether was removed and the acid distilled. It boiled at 222—224° (1.3 mm) with some decomposition. n_D^{20} 1.4858. It weighed 7.8 g; yield 65.5 %. The acid had a pale yellowish colour and was slightly impure. Many attempts to purify it by chromatography (alumina column) failed. It was therefore obtained pure *via* the S-(*p*-chlorobenzyl)-thiuronium salt.

S-(p-chlorobenzyl)-thiuronium salt. For the preparation of this salt the procedure described by Shriner *et al.*[30] was followed with the exception that the acid was dissolved in an ethanol-water solution and neutralized. From 6 g of acid 8.5 g of salt were obtained; m.p. 136—141°. Two recrystallizations from ethanol gave 7 g of pure derivative. m.p. 141—143°; yield 75 %. (Found: Se 14.12. Calc. for $C_{26}H_{45}ClNO_2SeS$: Se 14.00).

3-Selena-10-methyloctadecanoic acid was liberated from its S-(*p*-chlorobenzyl)-thiuronium salt by placing 6 g of the salt and 30 ml water in a clean separatory funnel, and adding excess 2 N hydrochloric acid. The clean oily product was extracted with distilled ether and dried over magnesium sulphate. The ether was removed and the acid was dried further under reduced pressure (1 mm) over a water-bath. The pure acid was oily, and weighed 3.2 g. n_D^{20} 1.4864; yield 82.7 %. (Found: Se 21.73; equiv. wt. 364. Calc. for $C_{18}H_{36}O_2Se$: Se 21.73; equiv. wt. 363.4).

3-Selena-10-methyloctadecanamide. 1.2 g acid were dissolved in 25 ml of dry benzene and to this mixture 3 ml oxalyl chloride were added. It was refluxed for 2 h. The excess oxalyl chloride and the benzene were removed and 50 ml cold concentrated ammonia were added to the acid chloride. It was shaken for 5 min and the solid material was filtered off and dried. It melted at 43—46° and weighed 0.9 g; yield 75 %. Three recrystallizations from carbon tetrachloride gave the pure amide, m.p. 45—47°. (Found: N 3.95; Se 21.69. Calc. for $C_{18}H_{37}NOSe$: N 3.87; Se 21.78).

B. 9-Selena-10-methyloctadecanoic acid

ω-Bromocaprylic acid. Diethyl azelate and azelaic acid were used for the preparation of the ethyl hydrogen azelate, according to the procedure described by Swann *et al.*[31] The half ester was converted to its ethyl ω-bromocaprylate following the method used by Allen and Wilson [31] and that of Hunsdiecker and Hunsdiecker.[32] This was hydrolyzed with a 48 % hydrogen bromide solution which furnished ω-bromocaprylic acid with m.p. 35—37°.

ω,ω'-Di-seleno-di-caprylic acid (IX). This diselenide was prepared from sodium diselenide and ω-bromocaprylic acid according to Fredga and Lindgren.[33]

9-Selena-10-methyloctadecanoic acid (X). Following the procedure previously described for the preparation of 3-selena-10-methyloctadecanoic acid, 8.9 g (0.02 mole) ω,ω'-di-seleno-di-caprylic acid were dissolved in 100 ml 2 N ammonium hydroxide solution and excess Rongalite was added until decolourization took place. 9.9 g (0.04 mole) of 2-bromodecane in 100 ml of ethanol were added to the solution. The reaction mixture was shaken for 24 h and allowed to stand for a day. The solution was diluted to 3 times its volume and acidified with 2 N hydrochloric acid which gave a yellowish suspension containing some elemental selenium. On standing in the refrigerator the material precipitated and was filtered off and washed with ice water. It was dried in a desiccator and became semisolid at room temperature. The semisolid material was dissolved in ligroin and some active charcoal was added to the solution, which was boiled and filtered. After

standing at room temperature, 1.5 g organic yellowish white material appeared, which was not possible to purify. The ligroin was evaporated and the remaining oily acid weighed 7.4 g; yield 50.8 %. It was crystallized twice from acetone at −50° and filtered off rapidly. The acid melted at 16−17.5°, n_D^{20} 1.4855. (Found: Se 21.46; equiv. wt. 364.5. Calc. for $C_{18}H_{36}O_2Se$: Se 21.73; equiv. wt. 363.4).

9-Selena-10-methyloctadecanamide. This amide was prepared according to the procedure already described for the preparation of 3-selena-10-methyloctadecanamide and gave a 75 % yield; m.p. 78.5−80.5°. (Found: N 4.06; Se 21.60. Calc. for $C_{18}H_{37}NOSe$: N 3.87; Se 21.78).

The selenium analyses were carried out according to Fredga.[34]

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