Table 2. The non-additive character of the activation of crystalline propionyl CoA carboxylase by K^+ and NH_4^+ .

Addition to the standard incubation mixture	Enzyme activity c.p.m. $^{14}CO_2$ fixed*
None	950
KCl 5 μ moles	1560
NH_4Cl 5 μ moles	1520
KCl 5 μ moles + NH_4Cl 5 μ moles	1500

* details of the assay in text.

planation can be offered for the observed discrepancy. The observation that the effects of the different univalent ions investigated vary greatly with their size suggests that steric factors may be involved in the metal activation effects.

The amounts of $^{14}CO_2$ fixed in these experiments are considerably smaller than what could be expected from stoichiometric considerations, using 0.025 units of the enzyme per standard incubation mixture. However, the specific activity and the kinetic constants of the enzyme have been determined (by Dr. Kaziro³) in a medium containing 150 μ moles K. It is evident from the results reported here that, without any or with only an insignificant concentration of potassium ions, the enzyme activity decreases to levels below the nominal one.

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1. Neujahr, H. Y. and Mistry, S. P. *Federation Proc.* **21** (1962) 239.
2. Neujahr, H. Y. and Mistry, S. P. *Acta Chem. Scand.* **17** (1963) 1140.
3. Kaziro, Y., Ochoa, S., Warner, R. C. and Chen-Jo-Yun *J. Biol. Chem.* **216** (1961) 1917.
4. Kaziro, Y. *Personal communication to the author.*
5. Halenz, D. R. and Lane, M. D. *J. Biol. Chem.* **235** (1960) 878.
6. Simon, E. J. and Shemin, D. *J. Am. Chem. Soc.* **75** (1953) 2520.

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Di-selena-straight-chain Fatty Acids

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The selena mono carboxylic acids with one selenium atom incorporated in the chain are described in the literature and they have been prepared by syntheses appropriate for selenides. One of the chief methods is the reaction of an alkyl halide with the salt of a selenol acid, and following this procedure many long-chain selena-fatty acids have been made at this Institute¹⁻³.

During the investigation of the long-chain selena-fatty acids it was decided to investigate the effect of incorporating a second selenium atom in the chain. Such compounds may also be of interest with regard to their biological and pharmacological properties.

Following the usual procedure for the preparation of this type of acid, it was decided to prepare an ω -hydroxy selena acid, halogenate the hydroxy group, prepare the corresponding diselenide and reduce the diselenide; the subsequent reaction with alkyl halide would give the di-selena fatty acid.

In the initial step, ω, ω' -diselena-diundecanoic acid⁴ was prepared and reduced by Rongalite, and by the addition of ethylene bromohydrine, 14-hydroxy-12-selena-tetradecanoic acid was obtained (m.p. 61–62.5°, yield: 61 %). During the second step, the halogenation of the hydroxy group by the hydrogen bromide or hydrogen chloride, either as gas or in solution and with or without heat, caused the appearance of yellow or orange crystals showing that an undesired side reaction had taken place.

In the preparation of a series of 12-selena fatty acids it was found that the reactivity of alkyl bromide with ω -selenol undecanoic acid is substantially greater than that of alkyl chloride and the yield is far better. It was therefore decided to take advantage of this difference in the halide reactivities and use a mixed dihalide. In this case trimethylene chlorobromide was used, which worked successfully according to the following scheme:

A small quantity of seleno-di-undecanoic acid was isolated during the preparation of (I), but it is uncertain if it is present as an impurity in the starting material or was formed during the reaction.

Experimental. Trimethylene chlorobromide was prepared according to Cloke *et al.*⁵

15-Chloro-12-selena-pentadecanoic acid (I) 15.84 g (0.03 mole) of ω, ω' -diseleno-diundecanoic acid were placed in a 500 ml flask and 225 ml of 2 N aqueous ammonia were added. After some shaking, some of the ammonium salt separated in the flask. 150 ml of ethanol were thus added to dissolve the salt. The solution was reduced with Rongalite and added, a third at a time, shaking for 2 h after each addition, to a solution of 11.8 g (0.07 mole) of trimethylene chlorobromide and 120 ml of ethanol in a 700 ml flask. Shaking was continued for an additional period of 12 h and left to stand over night. The solution was then diluted with water and acidified with 2 N hydrochloric acid, liberating an oily substance which solidified in the refrigerator. The precipitate was filtered off, dried and dissolved in warm ligroin. It was left to cool at room temperature and gave 3 g of seleno-di-undecanoic acid. This was filtered off, recrystallized twice from ligroin and three times from methanol yielding 2 g of pure substance, m.p. 105–106.5°. (Found: Se 17.71; equiv. wt. 226.2. Calc. for $\text{C}_{22}\text{H}_{42}\text{O}_4\text{Se}$: Se 17.57; equiv. wt. 224.7).

The filtrate was placed in the refrigerator and gave 12 g of 15-chloro-12-selena-pentadecanoic acid, m.p. 37–39°. A second recrystallization from ligroin with cooling in the refrigerator, gave 11.4 g (55.7 %) of glistening crystals of irregular flakes, m.p. 38.5–39.5°. (Found: Se 23.03; equiv. wt. 342.5. Calc. for $\text{C}_{14}\text{H}_{27}\text{ClO}_2\text{Se}$: Se 23.10; equiv. wt. 341.8).

ω, ω' -Di-selena-di-12-selena-pentadecanoic acid (II). 10.20 g (0.03 mole) 15-chloro-12-selena-pentadecanoic acid (I) were neutralized with 60 ml of 0.5 N sodium carbonate solution and placed in a three-necked 250 ml flask with a stirrer, a condenser and a dropping funnel. 2.37 g of selenium were dissolved in a solution of 1.8 g of sodium hydroxide. 2.5 g of Rongalite and 50 ml of water, by shaking for one hour. The three-necked flask was warmed in the water bath. The sodium diselenide solution was then added dropwise over a period of half an hour. The solution was further stirred for an hour. On cooling, the sodium salt of the diseleno acid was filtered off, recrystallized from ethanol, dissolved in hot water and the solution acidified. The precipitate was recrystallized three times from methanol and gave 6.75 g (58.7 %) of yellow crystalline material, m.p. 73–76°. (Found: Se 40.93; equiv. wt. 386.4. Calc. for $\text{C}_{28}\text{H}_{54}\text{O}_4\text{Se}_2$: Se 40.99; equiv. wt. 385.3).

12,16-Di-selena-octadecanoic acid (III). 2.57 g (0.033 mole) ω, ω' -di-selena-di-12-selena-pentadecanoic acid (II) were dissolved in 25 ml of 2 N aqueous ammonia and 30 ml ethanol. During the reduction with Rongalite which took about 5 min, the yellow colour of the solution became reddish and then again yellow before becoming colourless. 1 g (0.091 mole) ethyl bromide in 40 ml of ethanol was added to the solution, which was shaken over night. The solution was diluted with water, acidified, and the precipitate filtered off. It was then recrystallized three times from petroleum ether (b.p. 30–65°) yielding 2 g (72.5 %) of glistening crystals, m.p. 42–44°. (Found: Se 38.01; equiv. wt. 412.9. Calc. for $\text{C}_{18}\text{H}_{32}\text{O}_2\text{Se}_2$: Se 38.11; equiv. wt. 414.3).

12,16-Di-selena-heptacosanoic acid (IV). Following the above method this acid was prepared from ω, ω' -di-selena-di-12-selena-pentadecanoic

acid (II) and undecyl bromide, but yielded an insoluble salt. The salt was recrystallized from ethanol, dissolved in a 50 % ethanol-water mixture and acidified. It was recrystallized twice from petroleum ether, yielding 2.6 g (72 %) of glistening crystals, m.p. 67.5–68°. (Found: Se 29.17; equiv. wt. 542.3. Calc. for $C_{25}H_{50}O_2Se_2$: Se 29.21; equiv. wt. 540.6).

The selenium analyses were carried out according to Fredga⁶.

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1. Fredga, A. and Lindgren, A. *Acta Chem. Scand.* **15** (1961) 938.
2. Rebane, E. *Arkiv Kemi* **20** (1962) 205.
3. Fredga, A. and Golmohammadi, R. *To be published*.
4. Fredga, A. and Lindgren, A. *To be published*.
5. Clock, J. B. *et al.* *J. Am. Chem. Soc.* **53** (1931) 2794.
6. Fredga, A. *Studien über Selen-di-karbon-säuren und Diselen-di-karbonsäuren*. Diss. *Uppsala Universitets årsskrift* **1953**:5, p. 16.

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Heartwood Constituents of *Laburnum alpinum* Bercht. & Presl.*

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Laburnum alpinum Bercht. & Presl (syn. *Cytisus alpinus* Mill., Swedish "gull-regn") is a small tree. The wood of old specimens contains a large amount of brown heartwood and a very small amount of yellowish sapwood. The heartwood is fairly resistant to wood destroying fungi and, like pine heartwood, cannot be properly digested by the normal acid sulphite process.

Extraction of the wood with ether gave 5–8 % soluble products which partly separated in crystalline form on concentration of the extract. More of the same material was precipitated on addition of benzene. The total yield of crude solid

material was 3–4 %. Most of this product was fairly easily soluble in hot water (A) and in this way could be separated from a much smaller amount of less soluble products (B). Alternatively, the crude product was acetylated with acetic anhydride in pyridine and the dry crude acetate poured into boiling ethanol; part of it went rapidly into solution, leaving a less soluble product (D) which was collected by filtration. On cooling the hot ethanolic solution a product (C) precipitated. Acetylation of products A and B yielded acetates essentially similar to the acetates C and D, respectively.

Repeated crystallisation from ethanol of the C products furnished pure, dimorphous 3,4,3',5'-tetraacetoxystilbene,¹ m.p. 115° and 127–128°,² easily converted into the corresponding tetramethyl ether, m.p. 68–69°. The latter, on oxidation with permanganate, gave an excellent yield of acids which were esterified with diazomethane and then brominated. Alkaline hydrolysis of the bromination product gave 6-bromoveratric acid and methyl 2,6-dibromo-3,5-dimethoxybenzoate. This method is very useful for the separate isolation of the two aromatic nuclei of this stilbene.

Recrystallisation of the D-products *a*), from pyridine (or acetic acid) or *b*), from large amounts of ethanol gave two acetates: a colourless, dimorphous acetate, m.p. 165–167° and 203–206° (E) and a usually slightly yellow acetate (F), m.p. 200–203°. The latter acetate could be recrystallised from acetic anhydride but it was converted into E on prolonged heating with this reagent.

The evaporated mother liquors obtained according to method *b*) gave a product which after several crystallisations from ethyl acetate melted at 169–171° (G). Repeated recrystallisations of G from acetic acid yielded impure products of gradually increasing melting points. Finally, compound F was obtained.

Analysis of the compounds E, F, and G gave carbon and hydrogen values in agreement with the empirical formulae $C_{21}H_{16}O_8$, $C_{19}H_{14}O_7$, and $C_{19}H_{14}O_6OCH_3$, respectively. The acetyl estimations (alkaline hydrolysis, acidification and steam distillation) gave very varying results; E generally gave values corresponding to

* Part of this work was communicated at the Second International Symposium on Natural Compounds in Prague 1962.