Effect of ethylenediaminetetra-acetic acid. This probably functions by chelating the Mn^{2+} ions necessary for the *iso*citric enzyme (see Moyle & Dixon, 1956).

Little attention appears to have been paid to the anaerobic metabolism of the citric acid cycle intermediates by mitochondrial and other washed particulate preparations. It is perhaps surprising to find such a high consumption of tricarboxylic acid under strictly anaerobic conditions by these preparations since the mitochondria are the sole site of the terminal oxidase of the electron-transport chain. The extensive oxidoreductions that can occur under anaerobic conditions emphasizes the danger of assessing substrate removal by simple measurement of the respiration in its presence since there seems no reason to expect that the competition of the DPN oxidase for the reduced coenzymes would remove them all from interaction with other enzymes and substrates.

SUMMARY

1. Washed particles of rat liver consumed citrate anaerobically at about 50% of the rate occurring aerobically.

2. The products of the anaerobic reaction were on the average malate plus fumarate 10%, α-oxoglutarate 4%, glutamate 32% and succinate 34%. Other substances found were carbohydrate, lactate and β-hydroxybutyrate.

3. The proportions of the substances formed anaerobically from citrate varied with the amount of tissue added and concentration of di- and triphosphopyridine nucleotide and adenosine triphosphate. These three substances increased the citrate consumption whereas dithionite, hydroxylamine and ethylenediaminetetra-acetic acid decreased it and cyanide completely abolished it.

4. Glutamic acid was formed from the tissue preparations on incubation without substrate and was also formed from the citrate added as substrate.

5. The anaerobic reactions of citrate are discussed.

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The Identity of Natural and Synthetic ββ-Dimethylacrylylcholine

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The hypobranchial gland of the marine snail *Thais floridana* (Southern oyster drill) has recently been found to contain in relatively high concentration a biologically active ester, identified as ββ-dimethylacrylylcholine (Whittaker, 1957; Keyl, Michaelson & Whittaker, 1957). This paper describes the isolation of the naturally occurring ester as the chemically pure aurichloride, and presents additional evidence for the identity of the natural and synthetic compounds.

The aurichloride of the naturally occurring ester has almost the same melting point and gold content as the synthetic aurichloride and showed no depression of melting point when mixed with the

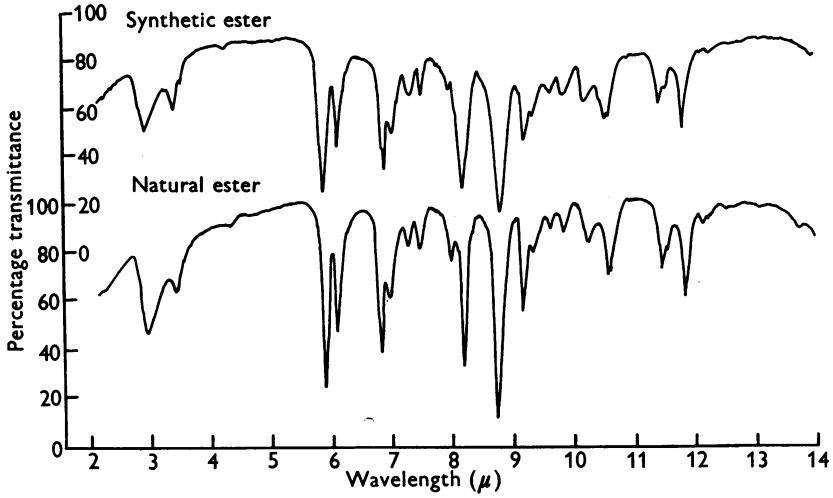

Fig. 1. Infrared-absorption spectra of the aurichlorides of synthetic and natural ββ-dimethylacrylylcholine.

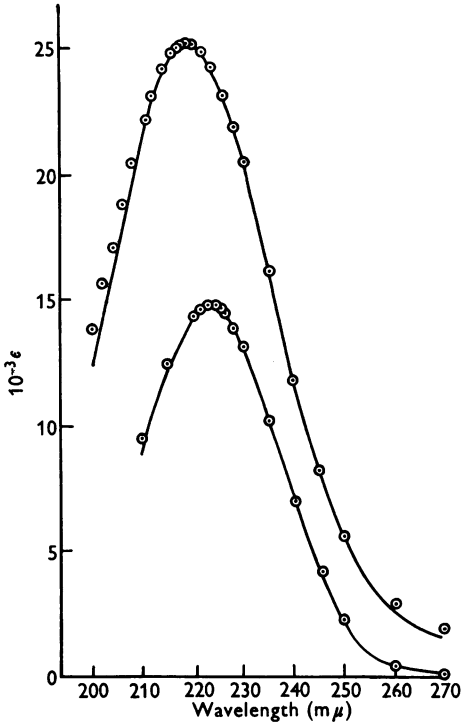

Fig. 2. Ultraviolet-absorption spectra of the aurichlorides (upper curve) and chlorides (lower curve) of synthetic (continuous line) and natural (⊙) ββ-dimethylacrylylcholine. Concentration of esters: 0·025 mM (aurichlorides); 0·05 mM (chlorides).

latter. The infrared (Fig. 1) and ultraviolet (Fig. 2, upper curve) absorption spectra of the two aurichlorides were also almost identical. In the infrared, bonds attributable to a carboxyl group and a C–C double bond in conjugation were detected at wavelengths of 5·84 and 6·03 μ respectively. The C–O–C band at 8·64 μ was also in evidence. The chlorides of the synthetic and naturally occurring esters, obtained by passing the aurichlorides through the anion-exchange resin De-Acidite FF, also had identical ultraviolet spectra (Fig. 2, lower curve), showing the typical absorption band of αβ-unsaturated fatty acid esters with an absorption maximum ($\lambda_{max.}$) at 223 mμ.

EXPERIMENTAL

Preparation of the naturally occurring ester

As aurichloride. Partially purified ester (580 mg.) prepared from snail hypobranchial extracts by reineckate precipitation as described by Keyl *et al.* (1957) was dissolved in water (4·2 ml.), and centrifuged to remove insoluble material and treated with excess of aqueous sodium aurichloride solution. The yellow precipitate was recrystallized twice from hot water, giving yellow needles (48 mg.) with m.p. 94° (Found: Au, 37·4. $C_{10}H_{20}O_2NAuCl_4$ requires Au, 37·5%). The mixed m.p. with the synthetic ester (prepared as described below) was 93–98°.

As chloride. The aurichloride (2·4 mg.) in water (1 ml.) was converted into the chloride by passing it through a column (10 cm. × 0·6 cm.) of De-Acidite FF (The Permutit Co. Ltd., London; −16 + 50 mesh) in the chloride form; 70% of the ester emerged in the first 4 ml. of effluent.

Synthesis of the ester

ββ-Dimethylacrylic anhydride. ββ-Dimethylacrylic acid (27 g., 0·27 mole) was refluxed with acetic anhydride (75 ml., 0·8 mole) for 24 hr. The ββ-dimethylacrylic anhydride was freed from acetic acid and acetic anhydride by repeated distillation *in vacuo*. The yield of light-yellow liquid, b.p. 82·5° at 0·5 mm. Hg, n_D^{25} 1·4866, was 12·5 g. (0·07 mole, 26%).

N-Dimethylaminoethyl-ββ-dimethylacrylate. The above anhydride (12·5 g., 0·07 mole) was refluxed overnight with dimethylaminoethanol (6·25 g., 0·07 mole); the reaction mixture was distilled *in vacuo* to give 15 g. of crude ester, b.p. 60–65° at 0·5 mm. Hg.

ββ-Dimethylacrylylcholine. A mixture of the above crude ester (10 g.) and methyl iodide (20 g., 0·14 mole) in dry ether (125 ml.) was allowed to stand for 3 days at 25°; the precipitate was collected by filtration and crystallized from ethanol to give *ββ-dimethylacrylylcholine iodide* as a light-yellow powder (m.p. 160°), yield, 5 g. (0·015 mole, 32% of dimethylaminoethanol) (Found, C, 38·1; H, 6·5; I, 40·4. $C_{10}H_{20}O_2NI$ requires C, 38·3; H, 6·4; I, 40·5%). The iodide (36 mg., 0·12 m-mole) in water (1 ml.) was converted into the *chloride* by passing it through De-Acidite FF as described above; 75% of the ester emerged in the first 5 ml. of effluent. This was treated with excess of sodium aurichloride to give a yellow precipitate, which on recrystallization from water yielded the ester *aurichloride* as yellow needles, m.p. 97°, yield 12 mg. (0·023 m-mole, 19%) (Found Au, 37·5. $C_{10}H_{20}O_2NAuCl_4$ requires Au, 37·5%). The chloride was also obtained from this salt by passing it through De-Acidite FF as described for the naturally occurring ester.

Spectroscopic comparison of esters

Infrared light. Infrared spectroscopy was carried out in a Perkin–Elmer double-beam recording spectrophotometer with the KBr-window technique. The window contained 0·34% of the ester aurichloride. The spectra of the synthetic and natural esters are plotted side by side in Fig. 1 and will be seen to be essentially identical.

Ultraviolet light. Ultraviolet spectroscopy was carried out in a Hilger Uvispek spectrophotometer. Dilutions were made under standard conditions in relatively large volumes to minimize errors due to absorption of the ester on glassware. All molar-extinction coefficients (ϵ) were calculated from the optical density of a 1 cm. thickness of solution and the molar concentration of ester, determined for the chlorides by the ferric hydroxamate method (Hestrin, 1949) at 520 mμ with the synthetic ester iodide as standard. The results are plotted in Fig. 2. It will be seen that the aurichlorides (upper curve) have a higher $\epsilon_{max.}$ and lower $\lambda_{max.}$ than the chlorides (lower curve), and that the spectra of the synthetic and natural esters are essentially identical.

SUMMARY

1. The biologically active choline ester, ββ-dimethylacrylylcholine, has been isolated from the hypobranchial gland of the marine snail *Thais floridana* as the pure, crystalline aurichloride.

2. The identity of the naturally occurring compound with the synthetic ester is demonstrated by physical and chemical methods.

The synthesis of ββ-dimethylacrylylcholine iodide was carried out by Dr L. E. Tammelin and Ing. I. Enander, and the infrared spectroscopy by Dr L. Larsson, Research Institute of National Defence, Department 1, Sundyberg 4, Sweden. I am most grateful to them for permission to include their results.

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The *in vitro* Enzymic Hydroxylation of Steroids

5. HYDROGEN TRANSPORT IN OX-ADRENOCORTICAL MITOCHONDRIA IN RELATION TO STEROID HYDROXYLATION*

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It has been shown by Grant & Brownie (1955) and by Sweat & Lipscomb (1955) that reduced triphosphopyridine nucleotide (TPNH) is required for the hydroxylation of steroids in the 11β-position by enzymes in ox-adrenocortical mitochondria. Subsequently, Grant (1956*a*) demonstrated that fumarate, added to enzyme reaction mixtures for steroid 11β-hydroxylation, effects triphosphopyridine (TPN^+) reduction according to the reactions: (1) fumarate → L-malate; (2) L-malate + TPN^+ → pyruvate + carbon dioxide + TPNH + H^+. Sweat & Lipscomb (1955) suggested the following sequence of reactions for the formation of TPNH: (3) Fumarate → L-malate; (4) L-malate + diphosphopyridine nucleotide (DPN^+) → oxaloacetate + DPNH; (5) DPNH + TPN^+ → DPN^+ + TPNH. Reaction (5) requires the presence of a pyridine nucleotide transhydrogenase, known to occur in certain animal tissues (Kaplan, Colowick & Neufeld, 1953), but not previously reported in the adrenal cortex. The presence of the transhydrogenase in the adrenal cortex was not demonstrated

* Part 4: Grant (1956*a*).