Preliminary Notes

PN 1190

Preparation of malonyl coenzyme A by thioester exchange

Previous investigators have reported chemical synthesis of malonyl coenzyme A[1-3]. The excellent method of LYNEN[1], although producing quantitative yields in the transesterification reaction, requires the multistep synthesis of *N*-caprylcysteamine. MENON AND STERN[2] have used direct esterification of malonyl dichloride with coenzyme A to produce yields of 50–60 % based on analysis of thioester formation. Monothiophenylmalonate prepared by the mixed anhydride method was employed by BRESSLER AND WAKIL[3] to produce yields of 40–50 % malonyl coenzyme A.

Malonyl monochloride was prepared by the following modification of previously reported methods[1,4]. Stoichiometric quantities (0.04 mole) of malonic acid and thionyl chloride were dissolved in 10 ml of anhydrous (dried over sodium) diethyl ether and refluxed for 2 h. During reflux, the mixture must be protected from air. The solvent was then removed under vacuum taking care that no heat be applied to the mixture. The resulting pale-yellow residue (yield 100 %) gave a melting point of 62–64° with decomposition (reported m.p. 64° (ref. 4), m.p. 62–64° (ref. 1)).

Mono-*p*-thiocresyl malonate was prepared by dissolving stoichiometric quantities (0.04 mole) of the monoacid chloride and *p*-thiocresol (Eastman Organic Chemicals) in 10 ml anhyd. diethyl ether. The reaction mixture was protected from air and was allowed to remain at room temperature overnight (18 h). Some fine crystals remained on the bottom of the reaction flask. A mixed melting-point determination showed these to be malonic acid crystals. The supernatant ether solution was extracted with 5% $KHCO_3$ and the aqueous phase was acidified yielding a precipitate of the thioester. The extraction was continued until no more precipitate was formed on acidification. The precipitates were taken up in diethyl ether after decantation of the supernatant aqueous phase. The ether volume was reduced under vacuum and the mixture placed at 4° overnight. The resulting crystals were recrystallized twice from chloroform–petroleum ether. The melting point was determined as 82–84°. [Calc. for $C_{10}H_{10}O_3S$: C, 57.12; H, 4.79. Found: C, 57.05, 56.95; H, 5.18, 4.98.] The yield of pure mono-*p*-thiocresyl malonate is 25–30 %.

The transesterification step to produce malonyl coenzyme A is a modification of the method of LYNEN[1]. 0.1 mmole $KHCO_3$ was dissolved in 2 ml distilled water. The mono-*p*-thiocresyl malonate (0.05 mmole) was weighed directly into this solution and dissolved. Coenzyme A (Pabst Laboratories; 5 μmoles) was weighed directly into the mixture. The reaction was allowed to progress at room temperature for 1 h at which time the solution is very cloudy due to formation of insoluble thiocresol. The reaction was then extracted with 10 ml diethyl ether for 2 h. At the end of this extraction, excess ether was removed with a pipet and the reaction mixture was acidified with Dowex-50W X4 (Baker) to pH 4.0. Diethyl ether was then employed as before to extract the reaction mixture at room temperature for 2 h. After decanting the

ether layer, nitrogen was bubbled through the reaction mixture until no further odor of ether was given off. The yield of malonyl coenzyme A so prepared was 78% as measured by CoA disappearance by the nitroprusside test. All of the thiocresol produced was removed by the extraction as shown by a negative nitroprusside test on a similarly treated blank solution containing 5 μmoles of thiocresol. The hydroxamic acid derivative of the malonyl coenzyme A was chromatographed according to the method of STADTMAN AND BARKER[5] and found to be identical to a similarly prepared malonyl monohydroxamate from analytically pure mono-*p*-thiocresyl malonate.

Further work is being done using other aromatic thiols which might give better yields of intermediate thioester or of malonyl coenzyme A.

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A new phosphoinositide containing four phosphates per inositol

In a previous study it was found that if the total lipid extract from brain cytoplasmic particulate fractions which had been incubated with ^{32}P under conditions of oxidative phosphorylation was chromatographed according to a method which separates the usual phosphatides[1], considerable amounts of radioactivity streaked[2]. This streaky material could, however, be resolved into discrete spots, termed A ($R_F = 0.06$), B ($R_F = 0.30$), and C ($R_F = 0.51$), if the lipid extract was chromatographed in phenol–ammonia on silicic acid-impregnated paper[2]. This communication deals mainly with the isolation and characterization of the water-soluble products of mild alkaline hydrolysis of the material in Spot B.

Spots B and C were not present in amounts sufficient to give a phosphorus stain on paper chromatograms, even when the chromatograms were maximally loaded with respect to total lipid present in the extracts. The spots were therefore routinely assayed by their radioactivity. It was found that Spots B and C were formed on incubation of brain-cortex slices in the bicarbonate saline of KREBS AND HENSELEIT[3] containing glucose and $^{32}P_i$.

10 kg of beef brain were homogenized with 0.3 N $HClO_4$, and the residue was serially extracted with a total of 40 l of ethanol – chloroform (1:1). After washing with 0.1 N HCl, the chloroform extracts were concentrated and the phospholipids were precipitated with acetone. The total yield of lipid at this stage was 258 g. The