Berg

7/12/56

Preparation of Adenyl-Methionine ≅ DCC

750 mg L-methionine and 350 mg ATP were ~~dissolved~~ mixed with 1 ml water and then added 4 ml pyridine followed by addition of 0.9 ml 8 M HCl. Almost all of the ATP dissolved but there was still methionine crystals visible to the solution (ice bath) added 5 gm DCC dissolved in small pyridine

Removed dose samples and added to 0.5 ml 1 N NH4OH Then added 1 ml $FeCl_3$ reagent filtered and read at 540 mμ.

Time	D_{540}	mM/ml using 0.340/μM methionine hydroxamic acid
15 min	305	18.0
45	630	37.1
60	910	53.5
130	910	53.5
150	950	55.8

At 60 min added 2.5 gm DCC as a solid

At 160 minutes volume of rx mixture was 18 ml
If above correct then 1.00 mM or completion.

At this point added 25 ml of H_2O (rm temp) stirred for 10 min Then filtered by suction (took about 20 minutes). Volume was 40 ml. Assayed for Hx

0.05 ml $D_{540} = .135 = 8\,\mu M/ml \times 40 = 320$ mM

Seems as if most of the material was degraded during the filtration etc

Berg

7/13/56

Reaction of L-methionine & DCC

.750 mg of L-methionine dissolved in 1.4 ml H_2O. Added 4 ml pyridine & then 0.9 ml 8 N HCl. Stirred in ice bath then added 5 gms DCC dissolved in 5 ml pyridine. 100k samples as before

Time	D_{570}
30'	018
60'	070
120	.057

Does not seem to be any significant amount of hydrolyzable reactive material in the donor or the acceptor (ASP)

7/14/56

Formation of Acetyl-L-methionine using less methionine

Meth / Asp = 2.5 / 1

110 mg Asp (0.3 mM) + 110 mg L-methionine (0.75 mM) mixed with 0.5 ml H_2O ~~[illegible]~~ Add 6.7 ml pyridine Then 0.12 ml 8N HCl Stir in ice bath Then add 2 gm of DCC in 2 ml pyridine.
Analyzed 0.05 ml by hydroxylamine as usual.

Time	Dsvo	µM/ml
30	0.367	21.6
60	0.461	27.2
180	0.782	46.0

Total volume of rx mixture
6.7 ml × 46.0 = 308 µM

Started = 317 µM Asp

At end of 5 hrs + 40 minutes took sample
at read 585 = 230 µM
Some breakdown.

7/16/56

Synthesis of Adenyl-L-methionine using Slight Excess of L-methionine

110 mg ATP (.31 mM) + 60 mg L-methionine (0.4 mM) mixed with 0.55 ml H_2O. Added 1.7 ml pyridine and then 0.06 ml 8N HCl. Stir in ice bath. Add 2 gm DCC in 2 ml pyridine.

Analyzed 0.05 ml aliquots ± NH_2OH + $FeCl_3$

Time	D_{540}	µM/ml	Total (vol 6.5 ml)
30'	.338	19.7	128
60'	.442	26.0	169
120'	.741	43.6	284
210'	.718	42.3	275

theory 310 $\quad 284/310 = 92\%$

Synthesis of Adenyl L-methionine ± Methionine Limiting

110 mg ATP (.31 mM) + 22 mg L-methionine (0.14 mM) mixed ± 0.55 ml H_2O. Added 1.7 ml pyridine, then added 0.06 ml 8N HCl. Stir in ice bath and add 2 gm DCC in 2 ml pyridine.

Analyzed as above

Time	D_{540}	mM/ml	Total.
45'	.137	8.1	53
105'	.393	23.1	150
150	.373	22.0	143

7/16/62

Summary:

Moles Meth/moles MM / moles MP	Ratio	Water/pyridine	Dec/meth	Yield	
5/1	5	2.3/9	25/5 = 5	100%	(based on AMP)
5/0	-	2.3/9	25/5 = 5	0	"
0.75/0.31	2.5	0.62/3.7	10/.75 = 13	97%	"
0.4/0.31	1.3	0.61/3.7	10/.4 = 25	92%	"
0.15/.31	0.48	0.61/3.7	10/.15 = 67	100%	(based on Meth)

7/13

Prep of Acetyl-Meth

350 mg Asp + 160 mg L-methionine mixed in 1 ml H_2O. Treated in exactly same way as described on 7/9. However at end instead added 60 mg methionine instead of 160.

1 min	D540	mM/ml.	Total based on volume 13 ml
60'	245		
120'	475		
180'	499	29.4	382 μM

Actually put in 400 μM

7/19/52

Synthesis of Adenyl Imethionine

350 mg AsP (free acid) 10 mM + 160 mg L-methionine (1.07 mM) mixed with 1.1 ml H2O + 3.5 ml pyridine. Added 0.15 ml 8 N HCl. Cool with ice bath & magnetic stirrer. Add 4 gm DCC dissolved in 4 ml pyridine (cooled in ice). Entire mixture stirred in ice-bath. Removed 0.05 ml samples & added to 0.95 ml 1 N NH4OH (pH > 7). Then added 1 ml Folin reagent & blue, filtered & read vs blank at 540 mμ.

Time	D 540	mM/ml
85'	.481	28.3
180'	.982	57.8
240'	1.050	61.8
270'	1.166	68.7

vol 13

At 300' added 100 ml of acetone at ca -20° & let set in ice bath for 5 min. Heavy coarse ppt settled out. Then was filtered by suction & the ppt washed 2x with 100 ml portions of 75:25 acetone : ether and then washed with ca 15 ml ether. The solid was dried & placed in vacuum dessicator for ca hour. Yield dry powder 650 mg

weighed out 49 mg & dissolved in 21 ml cold 0.0005 N HCl
filtered

Assay by Hx.

	D540	mM/ml	
~~0.05 ml~~			
0.10	379	11.1	} 11.1 x 21 = 233 mM/49 mg
0.20	758	11.1	

Spectrum on dilute acid solution

Diluted 0.03 → 10.0 ml with 0.01 M HCl.

λ	D
250	1.207
255	1.433
260	1.402
280	.278

250/260 = 0.2

250/260 = .86

Based on ratio of 0.22 $\bar{E}_{260} = 1.262 = .086$ mM/ml

= 28.6 mM/ml.

Then $\frac{11.1}{28.6}$ = 39% of AMP as Ad-Metl

Yield of AMP ppt was $2.1 \frac{28.6}{49} \times 650 = 797$ μM or ca 80 %

Preparation of DCC

Copied from Khorana notebook

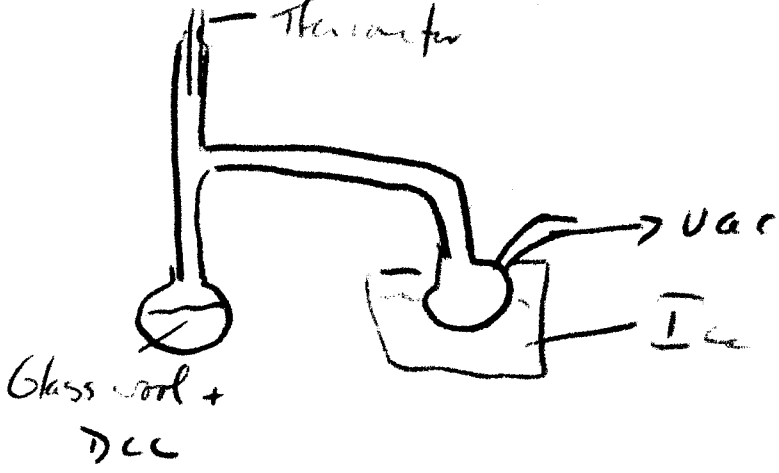
Prep of dicyclohexylthiourea

600cc hydrochloric acid to 400 cc ethyl alcohol. Add CS_2 (200 cc) carefully in very small portions and under constant shaking and cooling. After completion heat the almost solid contents for 1½ days till H_2S evolution is complete. The finely divided dithiocarbamate salt is extracted by chunky glass dicyclohexyl thiourea. Shook for 4 days and then filtered & washed with alcohol

$$C_6H_{11}NHC(=S)S^- + {}^+NH_3C_6H_{11} \longrightarrow C_6H_{11}NHCSNHC_6H_{11} + H_2S$$

Prep of DCC

50 gms of finely divided (powdered) dicyclohexylthiourea in ca 250-300 ml of CS_2 + 100g H_2O powder placed mechanically for ca 6-7 hours then filtered & washed with CS_2 in small amounts (also can use petether) Clear filtrate evaporate to oil. Dissolve in ca 150 ml of petether & dry over Na_2SO_4 anhydrous. Filter & leave petether. Distill in vacuo (a few mm at ca 100° Collect in steel air condenser



Yield when I did it was 25% but had been as high as 50-80%

7/23/56

Stability of the Crude Adenyl-Methionine

Dissolved 50 mg of the adenyl-methionine (crude 7/19) in 2 ml H_2O. pH was ~~3.2~~ 6 glass electrode

0.1 ml gave 0.350 O.D ~ MeOH + $FeCl_3$.

After 4 hours in ice bath 0.1 ml gave 0.352 ∴ No degradation occurred under these conditions.

Placed remainder at rm temperature and removed samples at various times

	D_{540}/0.1 ml
60'	.312
135'	.251
160'	~ .223 Becker very unsteady

Seems to be degraded about 10% per hour

On 8/2/56 dissolved 50 mg in 2 ml cold H_2O.

0.1 ml gave D_{540} .353

This material seems to be fairly stable In two weeks no breakdown.

Attempted Prep of Adenyl Methionine with di p-tolyl carbodiimide

55mg ATP (.16mM) + 30mg L-methionine (.20mM) mixed with 0.25ml H_2O. Then added 0.95ml pyridine followed by 0.03ml 3N HCl. Stirred in an ice bath. Added 1gm di-tolyl carbodiimide dissolved in 1ml pyridine. As soon as added the rx mixture solidified. Added ~4ml pyridine and some of the material dissolved the mixture becoming liquid. Removed samples at 1 & 2 hrs. There was no formation of methionine hydroxamic acid.

DO 440 0.002 & 0.005

One thing that appeared different was when an aliquot was added to the aqueous hydroxylamine there was a reddish brown ppt which separated out. Could this be the acyl urea of methionine + di-p-tolyl urea.

7/24/56

Prep of Adenyl-Methionine

350 mg ATP + 165 mg L-methionine mixed with 1.1 ml H_2O. Added 4.5 ml pyridine. Added 0.15 ml 8N HCl. Added 4 gm DCC dissolved in 4 ml pyridine. Stirred in ice bath. Removed 0.05 ml samples for assay.

Time	D_{540}	μM/ml	Estimated value of rx mixture 13-14 ml.
60'	.825	48.5	
165'	1.207	71.0	× 13-14 = 923-995 μM

At 180 minutes added 10 ml of acetone -20° and stirred for 1-2 minutes Then placed at -5° for ca 5 minutes. Filtered off by suction at rm temp. Took about 15 minutes to filter. Then washed with 60 ml of 70% acetone alcohol (-20°C) in small portions. Then washed with 30 ml of ether (-20°) and sucked partially dry. ~~Dissolved~~ Suspended material on funnel in 5 ml ice water by mashing then sucked dry. Repeated ē 5 ml more of cold H_2O ea Then washed filter with 1 ml H_2O.

Assay
0.05 ml — D_{540} .680 — μM/ml 40

Assayed again on 8/2/56
0.1 ml — D_{540} .735 — μM/ml 21.6
~ 50% degraded in ca 9 days.

Diluted 0.03 ml → 25 ml

λ	
250	1.204
253	1.438
257	1.550
260	1.415
265	1.325
240	.292

$250/260 = 0.85$

$260/260 = 0.21$

$1.329/14.7 \times 25/.03 = 75.3$ μM/ml

Correcting for pyridine content

$.292/.\cancel{X}2 = 0.22$

$40/75.3 = 53\%$

7/26/54

Prep of Acetyl-Meth put ppt ē 80% Alcohol 20% Ether

350 mg ASP + 165 mg L-meth mixed with 1.1 ml H_2O Then added 4.5 ml pyridine followed by 0.15 ml 3N HCl. Stirred in ice bath. Added 4 gm DCC in 4 ml pyridine. Removed 0.05 ml samples

Time	D_{280}	μM/ml.	
120'	1.130	66.5	69.3 × 13-14 = 900 - 970 or 90-97%
165'	1.175	69.3	

At 180 min added 110 ml of a 80:20 mixture of alcohol: ether (-15°C). Stirred in ice bath and after 5 min filtered ē portion. Complete in ca 5 min. Washed ppt above solvent on filter. Then dissolved ppt in 11 ml cold H_2O. All the ppt dissolved (the DC Urea must be soluble in the alcohol)

Assay vol 11-12 ml.

.03 → 25 ml 0.01 N HCl.

250	998	280/260 = .20	Concentration = $\frac{1.07}{14.3} \times \frac{25}{.03}$ =
255	1.202		
257	1.212		62.8 μM/ml
260	1.176	250/260 = .85	
280	.237		

Total

62.8 × 11-12 = 692 - 755 μM

Hydroxamic Acid

.05 ml	D_{540}	μM/ml.	Total
	.553	32.6	358 - 391

Purity $\frac{32.6}{62.8} = 52\%$

On 8/2 0.10 ml .545 16

About 50% left after 8 days. Kept frozen

7/27

Effect of HCl on Synthesis of Adenyl Methionine

55 g ASP + 30 g L-methionine mixed w 0.25 ml H2O. Added 0.85 ml pyridine stir in ice then add 0.9 gm DCC dissolved in 1 ml pyridine volume about 2.5-3 ml. Removed 0.05 ml samples

Time	D540	μM/ml	
65'	.155	9.1	
120'	.185	10.9	
200	.350	20.6	Total about 52-62 mM

Theory 160 mM

Thus in over three hours went about 30%. Seems as if the acid does speed up the reaction or maybe the rate of breakdown confined to the rate of synthesis is increased