there are (1) two 3-carbon monobasic acids, (2) one 2-carbon and one 4-carbon monobasic acid, (3) one 6-carbon dibasic acid. Allowing for the 4 or 5 mol. water formed, there are various possibilities, which would fit the equation perfectly. Speculation is here of little value, and characterization and determination of the fixed acids must be awaited.

SUMMARY

1. Inorganic phosphate or arsenate is required for the anaerobic oxidation of both pyruvate and α -ketobutyrate by ground preparations of pigeon brain with methylene blue as H-acceptor.

2. Inorganic phosphate stimulates the O_2 uptake and the utilization of pyruvate and of α -ketobutyrate by respiring preparations of ground pigeon brain (suspensions). Arsenate is slightly inhibitory.

3. α -Ketobutyrate is utilized at the same rate under comparable conditions by minced and ground preparations of pigeon brain. Pyruvate is more rapidly utilized by ground than by minced brain. This apparent discrepancy is discussed.

4. Adenine nucleotide markedly increases the

net O_2 uptake and the pyruvate utilization of pigeon brain suspensions when inorganic phosphate is present. It stimulates α -ketobutyrate oxidation under certain conditions, but normally its influence is difficult to detect.

5. Normally the O_2/α -ketobutyrate ratio and the R.Q. for α -ketobutyrate oxidation is the same for brain suspensions as for minced brain.

6. The O_2 /pyruvate ratio, R.Q. and CO_2 distribution as gas and bicarbonate have been determined in the presence and absence of added adenine nucleotide. For that part of the pyruvate oxidation specifically catalysed by adenine nucleotide, the following values have been obtained: O_2 /pyruvate ratio, 1.20; R.Q. 1.50; gaseous CO_2 /bicarbonate CO_2 , 2.0.

7. The mechanisms of pyruvate oxidation in minced and ground pigeon brain preparations are compared. The mode of oxidation of pyruvate by pigeon brain suspensions in the presence of inorganic phosphate and adenine nucleotide is discussed.

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The Biological Value of Mixed Proteins in Food Served in Royal Air Force Airmen's Messes

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While much information is available on the biological value of isolated proteins and of mixed proteins of individual foodstuffs, relatively little is known about the biological value of the proteins (nitrogen) of mixed human dietaries. Supplementary relationships between proteins make it naturally impossible to calculate such values from those of separate constituents. Hence an assess-

ment of a whole diet is of greater practical importance than the evaluation of the contribution of its different components.

The experiments described below were carried out to determine the biological value of the combined proteins contained in the food served to airmen at four Royal Air Force Stations in this country during October–December, 1941.

EXPERIMENTAL

At each Station the total food available to 5 airmen at all meals during 1 week was collected. Care was taken to ensure that this was representative of that served in the messes. The airmen queued for their meals and samples were obtained by taking at random 5 plates offered to the men as they filed past. The food was collected in galvanized pails. Tea and cocoa, as served, were not added, since the large amount of water would have caused difficulties during processing. The tinned milk, sugar and cocoa powder used in the making of the tea and cocoa, however, were added.

The food was then stored at -20° for 7 days. The total food was ground in the frozen state, the temperature not being allowed to exceed -5° . Care had to be taken to avoid the addition of large pieces of fat, and it was essential to spread the butter and margarine on the bread, otherwise these fats clogged the mill. The ground, frozen material which was snow-like in appearance was mixed and dried in an oven maintained at 70° and through which a rapid current of air was passed, for 6-7 hr. Except during the last $\frac{1}{2}$ hr., the temperature of the food was much lower than that of the oven, owing to the evaporation of water. The dried food was then minced in a sausage mincer and finally ground in a coffee mill; the resulting product was a fine brown-coloured powder, stable and suitable for analyses. The total weight of the dried food was determined and from it the average weight of dried diet representing one man's daily ration was calculated. The analyses of these dried diets for the total amounts of nutrients have been carried out and will be reported later.

The average amount of food left unconsumed was also ascertained. Table 1 gives the amount of protein in the food served and that in the food consumed. Protein values were obtained by multiplication of the nitrogen values by the factor 6.25.

Table 1. *Amounts of protein offered to and consumed by airmen (average/man daily)*

	Station 1 (diet I)	Station 2 (diet II)	Station 3 (diet III)	Station 4 (diet IV)
Protein in diet (%)	14.6	16.0	16.8	17.2
Protein offered (g.)	86.4	105.2	106.3	100.8
'Plate waste' (%)	3.3	6.0	5.3	7.8
Protein consumed (g.)	83.6	98.9	100.7	92.9

The amounts of the raw foodstuffs used in the different cookhouses were known. From tables of food values it was, therefore, possible to calculate the percentages of protein contributed to the dietaries by the individual foodstuffs. It was also possible to make a rough estimate of the amounts of the proteins which were of vegetable and animal origin respectively (Table 2).

The biological values and true digestibilities of diets from the four R.A.F. stations were determined by the method of Mitchell [Mitchell, 1924; Mitchell & Carman, 1926], using rats as experimental animals. The diets were diluted with starch to give an 8% level of protein intake ($N \times 6.25$), and sufficient margarine fat was added to bring the total fat content of each diet to 10%. As analysis indicated that the resulting diets were rather low in Ca, 1.5% $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ was added to each. Table 3 gives the composition and analysis of the experimental diets.

Table 2. *Percentages of total protein contributed by individual foodstuffs in dietary. Calculated total percentage of protein of animal origin*

Foodstuffs	Station 1	Station 2	Station 3	Station 4
Bread, white	22.8	25.4	22.0	20.1
Bread, National	6.7	10.8	6.9	8.1
Flour, white	5.2	4.2	4.1	4.2
Oatmeal	2.7	1.1	1.5	2.3
Rice	0.9	0.9	0.5	0.3
Custard powder	0.1	0.1	—	0.1
Shredded wheat	0.4	—	0.2	—
Cornflakes	0.3	1.2	0.5	0.2
Cake	3.1	—	3.4	—
Spongecake	0.9	—	—	—
Fruit pie	—	—	0.2	—
Jam	0.1	—	—	0.1
Milk	5.0	3.9	6.8	3.4
Cheese	4.2	2.8	2.7	3.9
Butter	0.1	—	0.1	0.1
Beef	11.1	14.8	12.3	5.6
Boneless beef	4.6	—	—	6.2
Mutton	—	3.0	—	4.9
Preserved meat	3.9	—	3.8	3.6
Liver	—	—	3.8	2.5
Kidney	2.6	5.3	2.5	—
Sausage	2.4	3.9	6.1	5.5
Oxtail	—	—	0.4	—
Oxheart	—	—	—	1.9
Meat roll	1.2	4.2	0.8	1.2
Meat paste	1.2	—	—	—
Meat pie	—	—	—	4.7
Pork pie	—	—	1.3	—
Cornish pastie	—	—	3.4	—
Sausage roll	—	—	1.3	—
Bacon	4.7	4.3	3.6	3.1
Fish (haddock)	3.8	4.0	—	5.5
Kippers	—	—	—	2.5
Fish cake	—	—	1.1	—
Potatoes	6.3	5.9	6.4	7.0
Cabbage	1.5	0.8	0.4	0.4
Brussels sprouts	—	—	0.2	0.4
Cauliflower	—	0.4	—	0.4
Carrot	0.4	0.2	0.3	0.5
Turnip	0.3	0.5	0.3	0.3
Parsnip	—	—	0.3	0.3
Celery	—	—	0.1	—
Beetroot	0.2	0.2	0.1	—
Onion	—	0.1	—	0.1
Lettuce	0.1	—	—	—
Tomatoes	0.1	0.1	0.1	0.1
Dried beans	1.3	0.5	1.4	—
Lentils	—	—	0.1	—
Canned beans	1.1	0.8	0.3	—
Apples	—	0.2	0.1	0.1
Prunes	—	0.1	—	—
Sultanas	0.2	—	—	0.2
Raisins	—	—	0.2	—
Cocoa	2.0	1.0	1.1	1.0
Percentage of total protein of animal origin (calc.)	43.2	42.2	42.7	44.7

Three groups of four litter-mate female rats 25-30 days old and weighing 52-71 g. were placed in glass and stainless steel metabolic cages. A description of the cages and of the experimental technique is given elsewhere [Henry, Kon & Watson, 1937]. Each animal received each of the diets in turn. The diets were fed mixed with $1\frac{1}{2}$ times their weight of water.

Table 3. *Composition and analysis of experimental diets*

R.A.F. diet no. ...	I	II	III	IV
R.A.F. diet (%)	54.7	49.9	47.8	46.5
Margarine fat (%)	1.6	1.1	1.6	3.2
Potato starch (%)	10.0	10.0	10.0	10.0
CaHPO ₄ ·2H ₂ O (%)	1.5	1.5	1.5	1.5
Rice starch (%)	32.2	37.5	39.1	38.8
Nitrogen (%)	1.329	1.313	1.308	1.297
Moisture (%)	10.96	10.62	10.46	10.83

RESULTS

The results of the rat tests are shown in Tables 4 and 5. All four diets have a high biological value and a high degree of digestibility. The differences between them are not large, the extremes for biological value being 77.6 for diet III and 81.2 for diet IV, and for true digestibility 85.1 and 88.5 for diets I and IV respectively. Statistical analysis

(Table 6) of the results by the paired *t*-test method ['Student', 1908; 1925] shows that the biological values of the proteins of diets II and IV are significantly superior to those of diet III but that other differences can be accounted for by errors of sampling. With one exception the order of ascending biological values is the same as that of ascending true digestibilities. When the two values are multiplied one by the other, one of the steps in calculation of the 'protein value' of a food [Mitchell, 1927; Mitchell & Hamilton, 1929], and the resultant figures are submitted to statistical analysis, diet IV again proves better than diet III but the other differences are without significance. When the 'protein values' of the four diets are calculated on the dry basis the following values are obtained: diet I, 9.5; diet II, 11.1; diet III, 11.1; and diet IV, 12.2. Here again diet IV proves best as it is highest in biological value, true digestibility and nitrogen

Table 4. *Individual nitrogen metabolism data*

Rat no.	R.A.F. diet tested	Wt. of rat, g.		Diet intake g.	N intake mg.	Faecal N mg.	Urinary N mg.	Biological value	True digestibility	Biological value × true digestibility
		Initial	Final							
Low egg-nitrogen diet										
1	—	62	73	49.33	—	90.6	98.0	—	—	—
2	—	70	72	43.08	—	109.5	87.1	—	—	—
3	—	66	71	41.65	—	92.3	95.0	—	—	—
4	—	69	76	50.62	—	109.6	88.4	—	—	—
5	—	63	66	39.37	—	96.2	81.6	—	—	—
6	—	62	68	49.64	—	107.6	87.3	—	—	—
7	—	58	65	45.84	—	91.1	75.4	—	—	—
8	—	59	66	47.50	—	103.0	80.8	—	—	—
9	—	60	67	48.74	—	100.3	61.2	—	—	—
10	—	58	62	43.35	—	98.3	68.3	—	—	—
11	—	55	61	44.13	—	94.1	68.6	—	—	—
12	—	55	63	48.38	—	100.0	68.6	—	—	—
First experimental period										
1	I	74	89	56.98	757.3	215.5	266.4	76.9	87.3	67.1
2	II	75	90	57.00	748.5	241.9	234.2	79.2	87.0	69.0
3	III	71	84	49.00	640.4	185.7	228.1	77.1	88.4	68.2
4	IV	81	94	57.45	745.1	204.1	222.3	82.1	90.0	73.9
5	I	70	77	39.98	531.5	170.7	196.0	76.8	86.6	66.4
6	II	70	83	53.93	708.1	219.8	212.6	81.2	86.5	70.2
7	III	70	82	54.49	712.5	220.1	225.8	78.1	84.7	65.4
8	IV	71	85	56.42	732.0	211.0	225.3	79.9	88.3	70.6
9	I	72	88	56.97	757.1	210.5	233.1	76.4	89.3	68.2
10	II	67	84	57.00	748.5	204.0	197.5	82.5	90.2	74.4
11	III	69	85	57.47	751.5	195.5	227.6	79.1	91.6	72.5
12	IV	72	89	57.46	745.3	202.7	203.3	82.6	89.8	74.2
Second experimental period										
1	IV	102	123	76.45	991.6	273.0	298.8	83.4	90.7	75.6
2	I	104	124	73.49	976.8	291.2	281.2	83.0	89.3	74.1
3	II	97	114	65.94	866.0	257.7	282.5	78.9	88.1	69.5
4	III	107	119	67.42	881.6	293.6	323.2	73.6	84.6	62.3
5	IV	85	102	59.87	776.5	232.2	221.6	83.7	89.5	74.9
6	I	95	110	71.96	777.5	285.6	252.5	80.4	69.7	56.0
7	II	95	110	66.47	873.0	240.4	246.0	84.6	88.4	74.8
8	III	95	108	65.95	862.3	271.2	301.1	75.5	85.9	64.8
9	IV	102	122	75.99	985.6	273.0	237.9	84.5	91.6	77.3
10	I	98	116	75.39	1002.0	318.1	245.6	83.1	85.6	71.2
11	II	99	116	72.97	958.0	323.0	248.8	82.7	85.1	70.4
12	III	101	118	76.00	993.9	288.6	285.3	79.9	88.9	71.1

Table 4 (cont.)

Table 1 (cont.)										
Rat no.	R.A.F. diet tested	Wt. of rat, g.		Diet intake g.	N intake mg.	Faecal N mg.	Urinary N mg.	Biological value	True digestibility	Biological value $\times$ true digestibility
		Initial	Final							
Third experimental period										
1	III	134	152	87.48	1144.0	347.8	405.3	78.1	89.6	69.9
2	IV	135	155	85.49	1109.0	332.9	344.2	81.7	89.6	73.2
3	I	125	143	78.47	1043.0	341.6	284.6	83.6	85.3	71.3
4	II	130	148	76.46	1004.0	285.9	293.2	83.9	90.1	75.6
5	III	112	132	68.60	897.0	282.5	259.5	84.2	88.1	74.2
6	IV	122	146	83.49	1083.0	297.0	308.4	83.9	92.3	77.5
7	I	120	128	60.16	799.7	248.4	280.2	81.6	85.0	69.4
8	II	129	136	73.48	965.0	309.2	235.8	77.2	85.6	66.1
9	III	131	153	86.99	1137.0	356.1	308.9	81.2	89.5	72.6
10	IV	127	150	86.99	1128.0	350.8	297.4	81.6	86.8	70.8
11	I	125	150	85.00	1130.0	336.6	283.5	84.5	90.1	76.1
12	II	127	151	87.50	1149.0	357.2	292.9	83.2	87.9	73.1
Fourth experimental period										
1	II	163	173	86.40	1135.0	439.5	403.1	78.6	83.3	65.4
2	III	164	179	93.87	1225.0	437.7	457.5	73.6	83.6	61.5
3	IV	150	164	84.51	1096.0	378.1	396.8	72.8	84.4	61.4
4	I	156	169	83.32	1107.0	375.9	390.2	76.1	85.1	64.8
5	II	137	152	76.49	1005.0	319.4	343.1	78.4	88.1	69.1
6	III	153	161	82.74	1082.0	367.9	409.7	73.4	86.6	63.5
7	IV	132	138	60.43	783.8	253.7	318.8	77.5	84.5	65.5
8	I	141	147	72.38	762.0	355.7	379.6	70.8	80.8	57.2
9	II	168	187	94.82	1245.0	435.1	352.3	80.4	87.5	70.3
10	III	162	183	97.45	1274.0	479.0	363.2	77.1	80.4	61.9
11	IV	163	180	96.00	1245.0	456.7	346.0	80.6	84.9	68.4
12	I	162	178	97.47	1297.0	419.9	372.3	79.1	87.5	69.2
Low egg-nitrogen diet										
1	—	170	164	46.86	—	148.0	189.3	—	—	—
2	—	173	172	56.77	—	144.3	181.6	—	—	—
3	—	161	167	68.42	—	171.2	132.5	—	—	—
4	—	169	170	60.92	—	160.2	164.2	—	—	—
5	—	146	140	36.29	—	96.4	143.3	—	—	—
6	—	157	153	49.44	—	139.5	145.2	—	—	—
7	—	133	142	59.85	—	135.0	175.3	—	—	—
8	—	143	149	60.76	—	147.9	145.7	—	—	—
9	—	178	170	52.35	—	164.9	128.8	—	—	—
10	—	182	179	58.92	—	140.6	147.1	—	—	—
11	—	183	181	64.44	—	193.1	131.9	—	—	—
12	—	181	182	70.87	—	195.9	129.1	—	—	—

Table 5. Mean data for biological values and true digestibilities of the diets studied

R.A.F. diet no.	Period I	Period II	Period III	Period IV	Mean	Standard error of mean
Biological value						
I	76.7	82.2	83.2	75.3	79.4	± 1.18
II	81.0	82.1	81.4	79.1	80.9	± 0.71
III	78.1	76.3	81.2	74.7	77.6	± 0.95
IV	81.5	83.9	82.4	77.0	81.2	± 0.95
True digestibility						
I	87.7	81.5	86.8	84.5	85.1	± 1.58
II	87.9	87.2	87.9	86.3	87.3	± 0.57
III	88.2	86.5	89.1	83.5	86.8	± 0.91
IV	89.4	90.6	89.6	84.6	88.5	± 0.80
Biological value $\times$ true digestibility						
I	67.2	67.1	72.3	63.7	67.6	± 1.74
II	71.2	71.6	71.6	68.3	70.7	± 0.94
III	68.7	66.1	72.2	62.3	67.3	± 1.34
IV	72.9	75.9	73.8	65.1	71.9	± 1.41

Table 6. *Statistical analysis of the data in Table 5*

Difference tested	Diet I-II	Diet I-III	Diet I-IV	Diet II-III	Diet II-IV	Diet III-IV
Biological value						
Numerical difference	-1.5	+1.8	-1.8	+3.3	-0.3	-3.6
Standard error of mean	± 1.11	± 1.58	± 1.77	± 1.23	± 1.18	± 1.23
P*	1:5	1:4	1:3	1:47	1:1	1:72
	Not sig.	Not sig.	Not sig.	Significant	Not sig.	Significant
True digestibility						
Numerical difference	-2.2	-1.7	-3.4	+0.5	-1.2	-1.7
Standard error of mean	± 1.66	± 1.65	± 1.97	± 1.34	± 1.06	± 1.16
P*	1:5	1:3	1:9	1:1	1:3	1:6
	Not sig.	Not sig.	Not sig.	Not sig.	Not sig.	Not sig.
Biological value $\times$ true digestibility						
Numerical difference	-3.1	+0.3	-4.3	+3.4	-1.2	-4.6
Standard error of mean	± 1.75	± 1.94	± 2.64	± 1.85	± 1.81	± 1.85
P*	1:10	1:1	1:8	1:11	1:2	1:33
	Not sig.	Not sig.	Not sig.	Not sig.	Not sig.	Significant

* P = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population.

content. Diet I takes last place, while the superiority in biological value of diet II over diet III is compensated for by the higher nitrogen content of III.

DISCUSSION

It is of interest to compare the data for the complete R.A.F. diets with the values reported in the literature for separate proteins, or for their combinations. Our results were obtained with young growing animals and therefore the combined requirements for growth and maintenance were measured. The work of Mitchell [1924] has shown that with growing rats the size of the animal has little effect on the estimate of the biological value of a protein. There are, however, indications [Sumner, 1938; French & Mattill, 1935; also unpublished work from one of our laboratories] that with adult rats the results are different from those with rats in the vigorous period of growth. Also, as the biological value of a protein varies according to the level of intake, only corresponding levels should be compared. The biological values of the R.A.F. diet proteins are high. Of single foodstuffs they are surpassed only by eggs and milk and nearly equalled by liver and kidney.* Of the proteins of vegetable products none can be said to attain these values. There must have been much supplementation of the poorer proteins by the better.

Relatively few estimations of combinations of foodstuffs have been published, and hardly any of them deal with complete mixed diets. Most come from India and China. Adolph & Cheng [1935] reported a very high biological value, 83 at a 10% level, for a mixture of maize, millet and soya bean, but Lan [1936] obtained a lower value, 73, for the same mixture. He quotes a value of 77, however,

* du Toit & Smuts [1941] have very recently reported a biological value of 94 for white fishmeal.

for a combination of soya bean and wheat gluten. Swaminathan [1938], using adult rats, investigated various mixtures of vegetable origin and obtained the highest values, 84 and 81 respectively, for combinations of red and green gram, *Cajanus indicus*, *Phaseolus radiatus* with ragi, *Eleusine coracana*. A mixed vegetarian diet of rice, red gram, black gram, amaranth leaves, brinjal and coconut yielded in his hands [Swaminathan, 1937] at a 10% level of intake, figures for biological value and true digestibility of 82 and 92 respectively. The supplementary relationship between animal and vegetable proteins is well known [Mitchell, 1924; Mitchell & Carman, 1926], and the value of an addition of milk has been confirmed by Swaminathan [1938]. He thus attained values as high as 84-94 for combinations of approximately 10 parts of ragi with 2 parts of various pulses and 1 part of dry skim milk, and values from 77 to 90 for similar combinations of rice, pulse and milk. There is no doubt that biological values as high and even higher than those now reported can be obtained for predominantly vegetarian diets. However, the 'protein value' of such diets works out at a much lower figure than those for the R.A.F. diets. Thus Swaminathan's [1937] diet, which is very similar to the 'Poor Madrassi' diet of Aykroyd & Krishnan [1937], contained only 10.5% crude protein as against nearly double this figure for the R.A.F. diets. Its 'protein value' is therefore 6.6—about half that of the best R.A.F. diet.

SUMMARY

1. A method is described whereby the total food served at meals may be converted into a homogeneous and stable product, suitable for analyses to determine its nutritive value.

2. The biological values of the mixed proteins contained in the food served at four R.A.F. Stations

were 79.4, 80.9, 77.6 and 81.2 respectively. The mixture of proteins in all diets had, therefore, a high biological value which must have been due to supplementation amongst the proteins. Not much more than 40% of the total proteins in the diets were of animal origin.

3. The true digestibility coefficients of the four diets were 84.5, 86.3, 83.5 and 84.6 respectively.

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Studies on the Acetone-Butanol Fermentation

4. ACETOACETIC ACID DECARBOXYLASE OF *CL. ACETOBUTYLICUM* (BY)

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Although the enzyme systems responsible for the fermentation of pyruvic acid and of glucose by *Cl. acetobutylicum* are so unstable as a whole that, in the absence of the substrate, their activity cannot be preserved in cell suspensions for more than a few hours [Davies, 1942], one enzyme has been found to be extremely stable; this is acetoacetic acid decarboxylase, the enzyme responsible for the decarboxylation of acetoacetic acid to acetone. The quantitative nature of the reaction, its optimum pH, and the effect of the age of the cells on their decarboxylase activity, have already been described; also the insensitivity of the enzyme to O₂, its ability to withstand acetone, and the fact that it can be extracted cell-free from the acetone powder [Davies, 1942]. In this paper the purification and properties of the isolated enzyme will be described. The parallel isolation of the lysine decarboxylase of *Escherichia coli* will be published later [Gale & Epps, 1943].

METHODS

Preparation of acetoacetic acid. 13 g. of redistilled ethyl acetoacetate were dissolved in 100 ml. N NaOH and kept at room temperature for 40 hr.

The ice-cooled solution was acidified with H₂SO₄ (25 ml. of 20% by vol.); the acetoacetic acid was twice extracted with 150 ml. ether and the ether evaporated at room temperature. From the residue neutralized with N NaOH the unchanged ester was removed by extraction with ether; finally the ether was evaporated *in vacuo*. After standardization by measuring the CO₂ evolved when a portion was incubated with acetone-treated cells of *Cl. acetobutylicum* in Warburg manometers, the concentration was adjusted to M/2.

Preparation of acetone-treated cells. 5 l. flasks containing 4½ l. of 2% glucose-liver medium [Davies & Stephenson, 1941] were sown with 80 ml. of a vigorously fermenting culture of *Cl. acetobutylicum* (BY) on 5% maize-meal mash, and were then incubated at 37° until the Rothera acetone test was intense (about 40 hr.). The culture was then strained through muslin and the cells collected in a Sharples super-centrifuge. They were washed once on the ordinary centrifuge with about 400 ml. distilled water and suspended in 50–100 ml. distilled water. This suspension was blown rapidly from a pipette into 10 vol. of vigorously stirred acetone; after a few minutes the precipitate was collected on a