Results. The results of the distribution experiments are given in Tables 1 and 2. It can be seen that ^{90}Y is more than 99 % extracted from 0.1 M HNO_3 with 0.1 M DBP in chloroform. Experiments with ^{89}Sr showed no activity in the organic phase under these conditions. However, with 1 M DBP small amounts of ^{89}Sr are extracted ($\log q = -2.216$). From this value we may calculate the extraction of ^{90}Sr to be less than 10^{-2} % ($\log q = -4.22$) with 0.1 M DBP.

Other measurements [11] have shown that DBP (HA) is highly dimerizised in chloroform. Within the concentration ranges of HNO_3 and DBP investigated here, the following equilibria may then explain the extraction of yttrium.

$$Y^{3+}\ (aq) + 3\ H_2A_2\ (org) \rightleftharpoons Y(HA_2)_3\ (org) + 3\ H^+\ (aq)$$

If the distribution of Y is given by

$$q = [Y(HA_2)_3]_{org}/[Y^{3+}]_{aq}$$

the equilibrium constant K will follow

$$\log K = \log q + 3 \log [H^+] - 3 \log C_A + 3 \log 2$$

where the DBP concentration in chloroform, $C_A = 2\ [H_2A_2]_{org}$.

The equilibrium constant K is calculated in the tables. The complex $Y(HA_2)_3$ is of a new type and will be further investigated. Other metal ions seem to form similar complexes [12].

Recommended procedure. a) Separation of ^{90}Sr and ^{90}Y. The sample is dissolved in 2—5 ml 0.1 M HNO_3 (HCl, $HClO_4$) and shaken with an equal volume of 0.1 M DBP in alcohol-free chloroform. The two phases are centrifuged and samples are withdrawn and evaporated to dryness. 80 % of the DBP in the chloroform sample may be removed at 190°C.

b) Preparation of carrier-free ^{90}Y. 5 ml of a 0.1 M HNO_3 solution with ^{90}Sr is shaken with 5 ml 0.1 M DBP in alcohol-free chloroform. The chloroform phase is then extracted with 5 ml of 5 M HNO_3. This nitric acid solution then contains 98 % of the ^{90}Y and 0.001 M of DBP. The DBP concentration can be lowered to 10^{-6} M, which is negligible for most purposes, by extraction first with 5 ml of chloroform and then twice with 5 ml of hexone (methyl *iso*butyl ketone). If carrier-free ^{90}Y is not wanted, inert yttrium may be added to the nitric acid solution and then precipitated with aqueous ammonia. This may be a convenient method of preparing an yttrium tracer, since neutron irradiated Y_2O_3 often contains some radioactive impurities.

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1. Herrmann, G. and Strassmann, F. *Z. Naturforsch.* **11a** (1956) 946.
2. Marathe, E. V. *J. Sci. Ind. Research India* **14B** (1955) 354.
3. Tompkins, E. R., Khym, J. X. and Cohn, W. E. *J. Am. Chem. Soc.* **69** (1947) 2769.
4. Salutsky, M. L. and Kirby, H. W. *Anal. Chem.* **27** (1955) 567.
5. Volchok, H. L. and Kulp, J. L. *Phys. Rev.* **97** (1955) 102.
6. Herrmann, G. and Strassmann, F. *Z. Naturforsch.* **10a** (1955) 146.
7. Lange, G., Herrmann, G. and Strassmann, F. *J. Inorg. & Nuclear Chem.* **4** (1957) 146.
8. Chetham-Strode, Jr., A. and Kindermann, E. M. *Phys. Rev.* **93** (1954) 1029.
9. Goldin, A. S. *U.S. AEC TID-7517* (1956) 323.
10. Scadden, E. M. and Ballou, N. E. *Anal. Chem.* **25** (1953) 1602.
11. Dyrssen, D. *Acta Chem. Scand. In press.*
12. Dyrssen, D. *To be published.*

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The Influence of Some Amino Acids on the Growth of and Vitamin B_{12} Production by *Streptomyces griseus* NzC5

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The purpose of this investigation was to study the influence of different amino acids on both the growth and the vitamin B_{12} formation of *Streptomyces griseus*.

Methods. The organism used for this investigation was a strain of *Streptomyces*

griseus, NzC5, which gives low yields of vitamin B_{12} and streptomycin.

The nutrient solution had the following composition: 0.5 g K_2HPO_4, $3H_2O$ – 0.5 g $MgSO_4$, $7H_2O$ – 0.015 g $CuSO_4$, $5H_2O$ – 0.015 g $MnSO_4$, $4H_2O$ – 0.03 g $ZnSO_4$, $7H_2O$ – 0.025 g $FeSO_4$, $7H_2O$ – 0.05 g $CaCl_2$, $2H_2O$ – 0.005 g $Co(NO_3)_2$, $6H_2O$ and 20 g glucose made up to 1 000 ml with distilled water. Different amino acids were added to the solution as the nitrogen source so that the nitrogen content of the medium was 0.60 to 0.66 g per litre.

The experiments were made either with 50 ml of nutrient solution in 250 ml Ehrlenmeyer flasks, shaken continuosly at 25°, or with 3 litres of nutrient solution in 5 litre fermentors at the same temperature. The results of these two experiments were in good agreement. The amount of mycelium and vitamin B_{12} was determined daily; the former by weighing the washed and dried mycelium and the latter microbiologically using *Escherichia coli*. Vitamin B_{12} was determined both in the mycelium and in the nutrient solution; most of the vitamin B_{12} was found to be present in the nutrient solution. The amount of nitrogen was determined periodically to ensure that an excess of assimilable nitrogen was present. All experiments were performed in duplicate.

Results. A number of experiments were made in which the nutrient solution contained only one amino acid as the nitrogen source. The amino acids used in these experiments were: arginine, aspartic acid, cystine, glutamic acid, glycine, *iso*leucine, leucine, serine and valine. The amounts of the amino acids used corresponded to 0.60 g nitrogen per litre nutrient solution. The maximum values for mycelium and vitamin B_{12} content are given in Table 1 where the amino acids are tabulated according to the growth obtained. Maximum growth was obtained after 4 to 6 days and the maximum vitamin content somewhat later, after the commencement of autolysis.

Only three of the nine amino acids tried maintained good growth, *viz.* glutamic acid, glycine and aspartic acid. The vitamin B_{12} formation, however, was not proportional to the growth obtained. Whereas the growth obtained with aspartic acid was almost as vigorous as that obtained with glycine, the amount of vitamin B_{12} formed with aspartic acid was much smaller (See column 4 which gives the ratio μg B_{12} : g mycelium).

Table 1. Growth and vitamin B_{12} formation with different amino acids.

Nitrogen source	Mycelium max. amount, g dry weight per litre	Vitamin B max. amount, μg per litre	Ratio $\frac{\mu g \text{ vitamin } B_{12}}{g \text{ mycelium}}$
Glutamic acid	4.5	400	88
Glycine	3.1	360	116
Aspartic acid	2.6	60	23
Arginine	0.4	10	25
Leucine	0.4	7	18
Valine	0.4	20	50
Cystine	0.3	15	50
*Iso*leucine	0.3	6	20
Serine	0.3	5	17

With regard to the other six amino acids only slight growth occurred. The vitamin formation was accordingly low as the presence of a certain amount of mycelium is a necessary condition for good formation of the vitamin. Two of these six amino acids, *viz.* valine and cystine, produced higher amounts of vitamin B_{12} than the other four. From these experiments it appears that no strict proportionality exists between growth and vitamin B_{12} formation.

The experiments summarized in Table 1 show that aspartic acid promotes good growth whereas the vitamin B_{12} production is small, calculated on g mycelium formed. Other amino acids maintain only weak growth, but the vitamin B_{12} formation is relatively high. In a series of experiments it was investigated whether aspartic acid together with the last mentioned amino acids would result in an increased vitamin B_{12} formation. In each experiment to one litre of nutrient solution 0.60 g nitrogen as aspartic acid and 0.06 g as one of the other amino acids was added. The total amount of nitrogen thus amounted to 0.66 g per litre. The fact that this increased amount of nitrogen is of no importance *per se* was demonstrated in an experiment in which the amount of aspartic acid was increased by 10 %.

While the addition of the amino acids had no marked effect on growth there was in most cases an increase in the vitamin B_{12} formation. In all cases except when β-alanine, arginine and leucine were used, the vitamin formation was increased by about

Table 2. Growth and vitamin B_{12} formation with aspartic acid + one of the following amino acids.

Amino acids added	Mycelium max. amount, g dry weight per litre	Vitamin B_{12} max. amount, μg per litre	Ratio $\frac{\mu g \text{ vitamin } B_{12}}{g \text{ mycelium}}$
β-alanine	3.3	60	18
Arginine	2.2	70	32
Glutamic acid	2.9	95	33
Glycine	3.0	100	33
Histidine	2.7	100	37
Leucine	2.9	80	28
Lysine	2.6	100	38
Proline	3.3	100	30
Serine	2.8	130	46
Tryptophane	2.7	95	35
Valine	2.4	100	42
No addition	2.6	60	23

50 %. Column 4, Table 2, shows that the greatest stimulation of the vitamin production was obtained with serine and valine (in the presence of aspartic acid). In the case of valine this is in good agreement with the experiments given in Table 1. Serine, however, when being the sole nitrogen source, was found to give a very low vitamin formation. The reason for this must be, that if serine is the only nitrogen source such a small amount of mycelium is formed that a high vitamin formation is impossible.

Glutamic acid and glycine have, when added in small amounts, a less marked effect on the vitamin B_{12} production than would be expected from Table 1 (if these amino acids constitute the sole nitrogen source they give rise to vigorous growth and vitamin production). The reason for this peculiar behaviour (Table 2) is probably that these amino acids, when added in small amounts, are used for the nitrogen assimilation and accordingly hardly anything remains for the formation of vitamin B_{12} which, as mentioned above, mainly takes place after the cessation of the growth. Serine and valine, on the other hand, are very bad nitrogen sources for growth and are thus not used during the growth phase but are left over to the vitamin forming phase.

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An Efficient Method for the Separation and Identification of Alkaloids in Biological Material

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The detection of alkaloids in biological material has long been based largely on colorreactions and crystal precipitation methods under the microscope. In recent years, paper chromatography has been employed to an increasing degree, pioneered by Munier *et al.*[1] and used by Jatzkewitz [2,3], Vidic [4] and others.

Buffered paper was employed by Carless and Woodhead [5], Goldbaum and Kazyak [6] and others. Schmall *et al.*[7] refined this technique by buffering zones to different pH values on the same paper. We have modified their technique for toxicological work and extended it to the identification of more than 30 compounds mainly of the alkaloid group. Quantitative estimation by spectrophotometry has also been worked out for many alkaloids. The basic principle of the method is as follows:

The tissue (whenever possible urine is employed, otherwise kidney, liver or blood) is heated with hydrochloric acid for 1 h for hydrolytic cleavage of adducts with glucuronic acid etc., and extracted in the usual way *via* amylacetate, 0.1 N HCl and chloroform.

The chloroform extract is dried, and evaporated to dryness. The residue is dissolved in hot 75 % ethanol and the ethanolic solution applied to a filter paper.

The migration and efficient separation of alkaloids and some other toxicologically important compounds on filter paper is achieved only if the free base is allowed to migrate in the direction of descending pH values and depends on the pH of the buffered zones as well as on the partition coefficients between the buffers and the mobile organic phase. The most efficient way to prepare the filter paper was the subject of extensive experimentation.

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