finally acidified with concentrated hydrochloric acid. The crude acid was collected on a filter and recrystallized first from 50% acetic acid and finally from benzene–petroleum ether (b. p. 90–100°). A product resulted which weighed 2.8 g. and melted at 150–152° (uncor.).

That this product is actually 5,6,7,8-tetrahydro-2-naphthoic acid was established by analysis, m. p. and mixed m. p., and by a comparison of the X-ray powder diffraction patterns of the authentic and unknown samples.

The above experiment has been repeated several times with consistent results.

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3-Amidinopyridine (Nicotinamidine)

BY H. J. BARBER AND R. SLACK

The failure of Bernthsen[1] to obtain 3-amidinopyridine by the interaction of nicotinonitrile and ammonium chloride, as cited by Krewson and Couch,[2] makes it desirable to publish details of the preparation of this amidine, accomplished some time ago in these laboratories.

Trypanocidal activity is developed in monoamidines on introduction of a second amidine group,[3] and the function of this may be merely to provide a second basic center. Though some of the monoamidines described by Easson and Pyman[4] fall into this class, the second basic group in these is a weak one. It was thought that a compound such as nicotinamidine would possess a second strongly basic center but in fact it titrated as a monoacid base, so that it gave no evidence on the point at issue.

In addition, the work of Fildes[5] has shown that the antibacterial action of the sulfanilyl drugs is probably due to competition with an enzymic metabolic process. Hence the synthesis of compounds with a close spatial resemblance to substances vital to, or associated with, bacterial growth, is of particular interest. The relationship between nicotinamide and nicotinamidine is sufficiently close to suggest that the latter might have possessed some anti-bacterial properties.

On treating nicotinonitrile[6] in excess ethyl alcohol with dry hydrogen chloride, a vigorous exothermic reaction occurred, the main reaction product being the hydrochloride of ethyl nicotinate. It was decided, therefore, to use an amount of alcohol only slightly in excess of that required by theory for the production of the corresponding iminoether hydrochloride. Following this procedure, the iminoether base was isolated as an oil which decomposed on attempted distillation at 8 mm. pressure. No further efforts were made to purify this compound, which reacted readily with ammonium chloride to give nicotinamidine hydrochloride. The amidine (1 in 4000 aqueous solution) was not active against *Staphylococcus aureus;* it was also found to be inactive against *T. equiperdum* infection in mice.

(1) Bernthsen, *Ann.*, **184**, 321-370 (1876).
(2) Krewson and Couch, THIS JOURNAL, **65**, 2256 (1943).
(3) Ashley, *et al.*, *J. Chem. Soc.*, 103 (1942).
(4) Easson and Pyman, *ibid.*, 2091 (1931).
(5) P. Fildes, *Lancet*, 1, 855 (1940).
(6) La Forge, THIS JOURNAL, **50**, 2477 (1928).

Experimental

3-Amidinopyridine.—3-Cyanopyridine (6.0 g.) was dissolved in dry chloroform (50 cc.) to which absolute ethyl alcohol (3.0 g.) had been added. This mixture was saturated at 0° with dry hydrogen chloride and allowed to stand at 0° for sixteen to eighteen hours. The viscous bottom layer which rapidly separated had by this time solidified. The whole was poured into ice-cold 50% sodium hydroxide solution (excess, final reaction alkaline to phenolphthalein), shaken vigorously, and the chloroform extract separated. This was then washed neutral with water, dried (potassium carbonate) and the solvent distilled to leave the crude iminoether base. This was dissolved in 75% ethyl alcohol (20 cc.) containing ammonium chloride (1.2 g.) and heated at 70° for four hours. After filtration (charcoal) a little ammonium chloride was removed after dilution of the liquors with acetone (2–3 vol.). The residual liquors were again diluted with acetone to cloudiness, when they were left to stand at 0° for several hours. 3-Amidinopyridine monohydrochloride separated in long slender colorless needles, m. p. 190°; yield 4 g.

Anal. Calcd. for $C_6H_7N_3 \cdot HCl$: N, 26.9; Cl, 21.97. Found: N, 26.1; Cl, 21.6.

The authors wish to thank Dr. R. Wien for carrying out the biological tests.

CHEMICAL RESEARCH DEPARTMENT
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DAGENHAM, ENGLAND RECEIVED MARCH 27, 1944

A Medium for Obtaining Maximal Growth Response in Microbiological Assays of Amino Acids

BY W. BAUMGARTEN, J. C. GAREY, MARY JEAN OLSEN, L. STONE AND C. S. BORUFF

In microbiological assays of amino acids the data given in support of the proposed media[1–6] demonstrate that practically no growth was observed when any one of the amino acids essential for the nutrition of the organisms was omitted. When an attempt was made to repeat the work of the above investigators, it was found that substitution of acid-hydrolyzed casein supplemented with cystine and tryptophan produced a greater growth response than when the casein hydrolyzate was replaced by a mixture of eighteen amino acids. The above results indicated that some stimulatory material was absent from the amino acid medium that was necessary for obtaining maximal growth.

The purpose of this paper is to show that maximal growth response with *L. casei* or *L. arabinosus 17-5* can be obtained when certain stimulatory nutrilites are added to a synthetic

(1) S. Shankman, *J Biol. Chem.*, **150**, 305 (1943).
(2) S. Shankman, Max S. Dunn and Louis B. Rubin, *ibid.*, **150**, 477 (1943).
(3) K. A. Kuiken, W. H. Norman, C. M. Lyman, F. Hale and L. Blotter, *ibid.*, **151**, 615 (1943).
(4) D. M. Hegsted, *ibid.*, **152**, 193 (1944).
(5) B. L. Hutchings and W. H. Peterson, *Proc. Soc. Exptl. Biol. Med.*, **52**, 36 (1943).
(6) S. Shankman, Max S. Dunn and Louis B. Rubin, *J. Biol. Chem.*, **151**, 511 (1943).

TABLE I

COMPOSITION OF MEDIUM FOR MICROBIOLOGICAL ASSAYS OF AMINO ACIDS

	L. casei and *L. arabinosus* Baumgarten, *et al.*	*L. casei* Hutchings,[5] *et al.*	*L. casei* Shankman,[6] *et al.*	*L. arabinosus* Kuiken,[3] *et al.*	*L. arabinosus* Hegsted[4]	*L. arabinosus* Shankman[1]
	Milligrams per tube of 10 milliliters					
Glucose	100	200	100	200	175	100
Sodium acetate	60	60	60	72.5	87.5	60
Adenine	0.5	0.2	0.2	0.05	0.04	0.1
Guanine	.5			.05	.04	.1
Uracil	.5			.05	.04	.1
Xanthine					.04	
K_2HPO_4	5	5	5	5	5	5
KH_2PO_4	5	5	5	5	5	5
$MgSO_4 \cdot 7H_2O$	2	2	2	2	2	2
$MnSO_4 \cdot 4H_2O$	0.1	0.1	0.1	0.1	0.1	0.1
NaCl	.1	.1	.1	.1	.1	.1
$FeSO_4 \cdot 7H_2O$	.1	.1	.1	.1	.1	.1
$(NH_4)_2SO_4$					30	
dl-Alanine	2	2	2	2	0.83	2
l(+)-Arginine	2	2	2	2	.83	2
dl-Aspartic acid	2	2	2	4	.83	4
l(−)-Cystine	2	2	2	2	.83	1
l(+)-Glutamic acid	2	2	2	4[a]	2.5	4
Glycine	2	2	2			
l(−)-Histidine	2	2	2	2	0.83	0.5
l(−)-Hydroxyproline	2	2	2		.83	
dl-Isoleucine	2	2	2	2	.83	2
l(−)-Leucine	2	2	2	2	.83	2
dl-Lysine	2	2	2	2	.83	2
dl-Methionine	2	2	2	2	.83	1
dl-Norleucine	2	2	2		.83	
dl-Phenylalanine	2	2	2	2	.83	1
l(−)-Proline	2	2	2	2	.83	
dl-Serine	2	2	2	2	.83	
dl-Threonine	2	2	2	2	.83	2
l(−)-Tryptophan	2	2	2	2	.83	0.33
l(−)-Tyrosine	2	2	2	2[a]	.83	.33
dl-Valine	2	2	2	2	.83	2
	Micrograms per Tube of 10 Milliliters					
Thiamin chloride	10			1	4	1
Pyridoxine hydrochloride	2	2	2	1	4	1
Calcium pantothenate	2	2	2	1	4	1
Riboflavin	2	2	2	2	4	2
Nicotinic acid	4	2	2	4	4	2
p-Aminobenzoic acid	2			0.005	4	0.1
Biotin	0.05	0.001	0.001	.004	0.004	.004
Tomato eluate				1 mg.		
Folic acid	4500[b]	.1	.1			

[a] The *dl* form was used. [b] Eluate Factor equivalent to Potency 1 material, for which we wish to thank Dr. R. J. Williams of the University of Texas.

medium. Maximal growth response was measured by the amount of titratable acid formed in seventy-two hours of incubation. At a one per cent. level of glucose and complete fermentation the calculated amount of lactic acid formed in 10 ml. of medium should be equivalent to 11.1 ml. of 0.1 *N* alkali. The data reported in this publication show that nearly 95% of the glucose was converted into the equivalent of lactic acid when a nutritionally complete medium was used. If maximal growth cannot be attained, stimulatory or inhibitory phenomena may occur and erroneous values result in assays of natural products. No difficulty is encountered in assays employing media which contain natural materials as acid hydrolyzed casein, chemically treated yeast extract or peptone. However, maximal growth frequently is not obtained in a synthetic medium since it is devoid of natural adjuncts which may contain essential or stimulatory nutrilites. In conducting assays employing a synthetic medium, the organisms should not show any appreciable

growth when an essential nutrilite is omitted, but on addition of this nutrilite maximal growth response should be observed.

The medium shown in Table I was adapted for use in this investigation. It is essentially the same as media previously reported[1-6] with the exception that thiamin and folic acid[7] have been added, thereby making the medium complete nutritionally for both *L. casei* and *L. arabinosus 17–5*. Thiamin acts as a stimulatory factor for the nutrition of *L. casei*, whereas folic acid stimulates the growth of *L. arabinosus*.

Table II demonstrates that maximal growth response is not obtained when previously proposed media for *L. casei*[5] or *L. arabinosus*[1] are employed. When casein hydrolyzate supplemented with cystine and tryptophan replaced the synthetic amino acids as the source of nitrogen, 90–95% of the maximal growth occurred. The medium *L. casei*[5] supported a growth response of 60 to 70% which was increased by the addition of thiamin to 90 to 95%, whereas folic acid increased the growth response of *L. arabinosus*[1] from 70 to 80% to 90 to 95%.

TABLE II

GROWTH RESPONSE OF *L. Casei* AND *L. Arabinosus* 17-5 IN VARIOUS CULTURE MEDIA

Medium	Amount of 0.1 *N* alkali required to neutralize 10 ml. medium after seventy-two hours of incubation: *L. casei* ml.	*L. arabinosus* ml.
1. Hutchings and Peterson[a]	7–8[b]	
2. Hutchings and Peterson[a] amino acids replaced by casein hydrolyzate supplemented with tryptophan and cystine	10–10.5	
3. Shankman[1]		7– 8.5
4. Shankman,[1] amino acids replaced with casein hydrolyzate supplemented with tryptophan and cystine		10–10.5
5. Proposed medium: 2 mg. each of 20 amino acids per tube	10–10.5	10–10.5
6. Same as No. 5, thiamin omitted	7–8	
7. Same as No. 5, folic acid omitted		8–9

[a] 1% glucose and 0.6% anhydrous sodium acetate used. [b] Theoretical acid production corresponds to 11.1 ml. of 0.1 *N* NaOH.

When the medium outlined in Table I was used in amino acid assays, no appreciable growth occurred when an essential amino acid was omitted (Blank titration values of 0.7 to 2.0 ml. of 0.1 *N* alkali). When the same essential amino acid was added in the proper concentration, maximal growth response was obtained.

(7) B. L. Hutchings, N. Bohonos and W. H. Peterson, *J. Biol. Chem.*, **141**, 521 (1941).

HIRAM WALKER & SONS, INC.
PEORIA, ILLINOIS

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Xylitol Esters of Fatty Acids

BY J. F. CARSON, JR., AND W. D. MACLAY

A series of fatty acid esters of the pentahydric alcohol, xylitol, has been prepared in connection with a study of plasticizers. The only esters of xylitol recorded in the literature are the pentaacetate[1] and the pentanitrate.[2]

Xylitol was prepared by high-pressure catalytic hydrogenation of D-xylose. The esters, xylitol pentapropionate, pentabutyrate, pentalaurate, pentamyristate, pentapalmitate, and pentastearate, were prepared by reaction of xylitol with the corresponding acid anhydride or acid chloride in pyridine. Xylitol pentapropionate and pentabutyrate are oily liquids with interesting possibilities as plasticizers for cellulose esters.[3] The xylitol penta-esters of lauric, myristic, palmitic and stearic acids are low-melting waxy solids. These latter esters could not be saponified completely with the customary ethanol–potassium hydroxide reagent; after refluxing for six hours, hydrolysis was only 50% complete. Saponification with a solution of potassium hydroxide in *n*-butyl alcohol for two hours gave satisfactory saponification equivalents. Data on the new xylitol esters are recorded in Table I.

TABLE I

PHYSICAL AND CHEMICAL CONSTANTS OF XYLITOL ESTERS

Ester, xylitol	M. p., °C.	d^{25}_4	$n^{25}D$	Saponification equivalent: Calcd.	Found
Pentapropionate	.....	1.1176	1.4424	86.4	86.8
Pentabutyrate	.....	1.0606	1.4436	100.4	100.1
Pentalaurate	33.5–35	....	....	213	214
Pentamyristate	45.5–47	....	....	241	239
Pentapalmitate	56–58	....	....	269	270
Pentastearate	66–68	....	....	297	295

Experimental

Preparation of Xylitol Pentapropionate and Pentabutyrate.—These esters were prepared by reaction of 15.0 g. (0.099 mole) of dry xylitol with 0.61 mole of the corresponding anhydride in 100 g. of pyridine. The reaction mixture was heated on a steam-bath for three hours and the ester isolated as an oil on pouring into a liter of chopped ice. The aqueous mixture was extracted with benzene or toluene, and the extract was washed successively with 3% sodium carbonate solution, 3% hydrochloric acid, and distilled water. Removal of the solvent *in vacuo* yielded the esters as pale amber liquids in 90–95% yields. Xylitol pentapropionate and pentabutyrate were purified by evaporative distillation in a short-path still of a type described by Matchett and Levine[4] at 0.1 mm. and a bath temperature of 120–150°. The distillations proceeded without noticeable decomposition to give almost quantitative yields of colorless product.

(1) Hockett and Hudson, THIS JOURNAL, **57**, 1753 (1935).
(2) Bertrand, *Bull. soc. chim.*, [3] **5**, 556, 740 (1891).
(3) Elam, Preusser and Page, *Modern Plastics*, **20**, No. 9, 95 (1943).
(4) J. R. Matchett and J. Levine, *Ind. Eng. Chem., Anal. Ed.*, **15**, 296 (1943).