940 mg (0,4 %) kristallines "Ibamarin", das mit unserem früheren Vergleichsmaterial identisch war. Die vereinten Mutterlaugen gaben nach erneuter Chromatographie weitere 50 mg der Verbindung mit Fp. 229–232°, deren Gesamtausbeute somit 0,1 % betrug.

"Ibamarin". "Ibamarin" wurde aus Benzol mit einem Zusatz von etwas Wasser umkristallisiert und analysenrein erhalten, Fp. 148–150°. Mischschmelzpunkt mit Cucurbitacin I (Fp. 148–150°) ergab keine Depression. Die IR-Spektren in Nujol und Chloroform waren identisch.

Substanz Fp. 234–237°. Der kristalline Stoff, Fp. 229–232°, wurde aus Benzol umkristallisiert, wobei farblose Blättchen, Fp. 234–237°, erhalten wurden, $[\alpha]_D^{22} - 60°$ ($CHCl_3$, *c* 1 %). (Gefunden: C 68,99; H 7,77; O 22,87. Berechnet für $C_{32}H_{44}O_8$: C 69,04; H 7,95; O 22,99). Der Mischschmelzpunkt mit Cucurbitacin E (Fp. 235–237°) gab keine Depression. Die IR-Spektren in Nujol und Chloroform waren identisch.

Dünnschicht-Chromatographie. (*a*) Cucurbitacin E, (*b*) Cucurbitacin I, (*c*) "Ibamarin", (*d*) "Acetyl-Ibamarin", und (*e*) Substanz Fp. 234–237°, wurden in 5–20 μg-Mengen auf Glasplatten mit Kieselgel G-Merck in üblicher Weise dünnschichtchromatographisch entwickelt. Als Fliessmittel diente Chloroform mit 5 % Äthanol-Zusatz. Die Flecken wurden durch Besprühen mit äthanolischer $FeCl_3$-Lösung (rotbraune Flecken) oder mit einem Gemisch aus 2 Teilen konz. H_3PO_4 und 8 Teilen Äthanol (graubraune Flecken nach Erhitzen auf 140°, mattgelbe Fluoreszenz unter UV-Licht) sichtbar gemacht. R_F-Werte: (*a*) und (*e*) 0,41, (*b*) und (*c*) 0,23, (*d*) 0,37.

Wir danken Dr. R. W. King für die Aufnahme der NMR-Spektren.

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Received July 17, 1962.

Production of some Amino Acids and B-Vitamins by the Corn Smut Fungus *U. maydis* (DC) Cda.

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During recent years we have studied some of the factors influencing the biosynthesis of lysine and threonine by *U. maydis* (DC) Cda. as well as the ability of this organism to utilize various sources of carbon and nitrogen for growth and amino acid production. [1,2] As a sideline to this work, we have also made a limited number of observations on the ability of *U. maydis* (DC) Cda. to produce valine and glutamic acid as well as a few B-vitamins when cultivated on different media in 10-litre fermentation vessels. It should be emphasized that this investigation was of an exploratory nature only and that no attempt was made to obtain maximal yields for the different substances studied.

A culture of *Ustilago maydis* (DC) Cda. was purchased from the Centralbureau voor Schimmelcultures, Baarn, Holland. This culture is identical with *U. maydis* ATCC 11427, P.R.L. 119 and N.R.R.L. 2321. It was maintained on agar slants, prepared by adding 1.5 % agar to the medium suggested by Dulaney.[9]

Four different media were used. Medium I was a synthetic medium and had the composition given in Table V, Ref.[2] Maltose (100 g/l) and a mixture of ammonium sulphate (6.0 g/l) and urea (1.8 g/l) were used as sources of carbon and nitrogen. It also contained all the mineral constituents that were found to be necessary for the growth of *U. maydis* (DC) Cda. Medium II was made up entirely from unhopped lager wort, which was diluted 1:1 with distilled

water. The carbohydrate content of the final medium was approximately 100 g/l, 80 % of which was fermentable with *Saccharomyces cereviseae*. The concentration of assimilable nitrogen was approximately 0.6 mg/ml. No minerals were added to this medium. Medium III contained beet sugar molasses as the carbon source and diammonium phosphate (10 g/l) as the nitrogen source. By dissolving 208 g of molasses in one litre of distilled water, a sugar content of about 100 g/l medium was obtained. No mineral constituents were added. Medium IV was identical with medium III but the supernatant fluid from the anaerobic digestion tanks for sewage sludge was used instead of water for the dilution of the molasses. The supernatant fluid from the sewage works was freed from particles before being used.

The organism, in all cases reported here, was grown in 10-liter fermentation vessels. The rate of aeration was 0.3 l per litre medium per min and the broth was stirred at about 700 r.p.m. The temperature was kept at 27°C. Inocula were prepared by growing the organism in shaking flasks at 25°C for 48 – 72 h.

Samples for analysis were withdrawn immediately after inoculation of the tanks and after 10 and 25 days. Each sample was incubated at 45°C for 24 h at pH 4.0 as this had been found to give maximal values for lysine and threonine. Before being submitted to the analytical procedures the incubation mixture was centrifuged to remove cell debris.

Microbiological methods were used for the estimation of the amino acids and vitamins under study. Lysine and glutamic acid were determined according to the tube assays of Steele *et al.* using *Leuconostoc mesenteroides* P-60 (ATCC 8042) as test organism [3]. Threonine and valine were also estimated by the procedures outlined by Steele *et al.* but in these cases *Streptococcus faecalis* ATCC 8043 was employed as test organism. For all amino acid determinations we used Difco's Bacto Assay Media. Growth was measured turbidimetrically after 24 h at 37° C in a Coleman Model 11 Universal spectrophotometer at 640 mμ.

Riboflavin was released by HCl treatment as described in the method of analysis of the *Association of Official Agricultural Chemists*, Eigth Edition [4]. *L. casei* ATCC 7469 served as test organism in the Difco's Bacto Riboflavin Assay Medium. For this as for the other vitamins cup plate assays were applied. The agar plates were incubated overnight at 37°C.

Niacin was also set free according to the method recommended by A.O.A.C., by autoclaving in 1 N H_2SO_4. The test medium was that of Bolinder and Larsen [5] using *S. faecalis* ATCC 8043. Niacin was used as standard.

An estimate of the total folic- and folinic acid activity was obtained in the following manner. The pH of the samples was adjusted with 1 M K_2HPO_4 to 6.5. To 10 ml samples was added 80 mg of the Bacto chicken pancreas conjugase preparation sold by Difco Laboratories, Detroit, USA. The mixture was then incubated under toluene overnight at 37°C. It was thereafter boiled for 5 min and centrifuged. The total folic- and folinic acid activity was estimated with *S. faecalis* ATCC 8043 as test organism. The same medium was used as for the niacin assay with the omission of folic acid and the addition of 1 mg of niacin per l medium (single strength). It should be pointed out that since the samples probably contained several members of the folic- and folinic acid group of factors, whereas pteroylglutamic acid served as standard, this assay merely gives an indication of the presence of large or small amounts of folic- and/or folinic acid activity. It does not give an estimate of the content of pteroylglutamic acid content of the samples.

The biotin assay was preceeded by autoclaving the samples in 1 N H_2SO_4 at about 120°C for 30 min. After neutralization the samples were assayed for biotin using *L. fermenti* ATCC 9338 as test organism. The basal medium was the same as the acetate medium described by Bolinder and Larsen [5] with the modifications mentioned by Sjöstedt and Ericson [6].

Vitamin B_{12} (cyanocobalamin) and related growth factors were released by boiling in dilute KCN at approximately pH 5 for 15 min. *E. coli* 113 – 3 was used as test organism in the medium suggested by Diding [7] but modified by the addition of 100 mg thiomalic acid and 10 mg KCN per litre medium (single strength). Cyanocobalamin served as standard.

The results are summarized in Table 1. For the sake of brevity only the initial values and the highest or lowest values observed during the fermentation are presented. It should be mentioned that the growth in medium I was poorer than previously encountered and that the values for lysine and threonine are far below those reported earlier. The data from this experiment are nevertheless included as they clearly demonstrate the ability of *U. maydis* to produce all the vitamins tested with the exception of vitamin B_{12}. We have been unable to detect any ability of *U. maydis* to synthesize this vitamin (or related factors) which is contrary to the report by Haskins *et al.* [8] In fact the B_{12}-activity present in medium IV which contained sewage sludge was utilized by the organism in a few days. In general the

Table 1. Concentration of some amino acids and vitamins in incubated (45°C, pH 4.0) *U. maydis* broth. The figures within brackets give the number of days of fermentation.

Medium	Cell number × 10^6	pH	Amino acids				Vitamins					
			Lysine	Threonine	Valine	Glutamic acid	Ribo-flavine	Niacin	Biotin	B_{12}-activity	Folic- and folinic acid activity direct treated	
			μg/ml	μg/ml	μg/ml	μg/ml	mμg/ml	mμg/ml	mμg/ml	mμg/ml	mμg/ml	mμg/ml
I	11.2(0)	6.5(0)	10(0)	10(0)	25(0)	10(0)	10(0)	35(0)	5(0)	0(0)	6(0)	3(0)
	325(5)	8.2(5)	50(5)	50(5)	50(2)	80(5)	75(5)	120(5)	12(5)	0(5)	150(10)	190(10)
II	15.0(0)	6.4(0)	100(0)	100(0)	140(0)	60(0)	25(0)	320(0)	10(0)	25*(0)	30(0)	75(0)
	1150(10)	5.4(10)	310(5)	250(5)	210(2)	110(2)	450(10)	470(5)	80(10)	70*(5)	100(5)	130(5)
III	24(0)	7.1(0)	35(0)	100(0)	180(0)	250(0)	30(0)	560(0)	6(0)	10*(0)	20(0)	20(0)
	900(5)	6.3(5)	35(5)	40(5)	90(5)	190(5)	75(5)	560(5)	5(0)	5*(5)	130(5)	130(5)
IV	24(0)	7.1(0)	35(0)	100(0)	200(0)	280(0)	30(0)	380(0)	30(0)	35(0)	20(0)	20(0)
	750(10)	5.6(10)	70(10)	80(10)	110(10)	320(5)	180(10)	560(2)	40(5)	7*(10)	75(5)	380(10)

* Diffuse growth zones not due to B_{12}-like factors but more likely to methionine.

concentrations of the vitamins present at any stage of the fermentation were low for all media. Also the ability to synthesize lysine, threonine, valine and glutamic acid was poor under the conditions of this experiment.

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Received August 7, 1962.

Crystal Structure Data for the Compounds $TaCl_5 \cdot POCl_3$ and $TiCl_4 \cdot 2POCl_3$

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Preliminary studies of the compounds $TaCl_5 \cdot POCl_3$ and $TiCl_4 \cdot 2POCl_3$ have been made as part of an investigation of the crystal structures of addition compounds formed between metal halides and $POCl_3$ or $PO(CH_3)_3$. Single crystals were prepared in sealed capillary tubes using a zone melting technique. Rotation and Weissenberg photographs (layer-lines 0—2) were taken with CuK radiation. From the space group and the approximate unit cell dimensions thus obtained and from a comparison of the intensities it was found that the compound $TaCl_5 \cdot POCl_3$ is isostructural with $SbCl_5 \cdot POCl_3$ [1] and the compound $TiCl_4 \cdot 2POCl_3$ is isostructural with $SnCl_4 \cdot 2POCl_3$. [2]

No further work on these compounds is intended at present.

I wish to thank Professor G. Hägg for all the facilities placed at my disposal and Professor I. Lindqvist for continued interest and stimulating discussions. This work has been sponsored by a grant from Air Force Office of Scientific Research, OAR, through the European Office, Aerospace Research, United States Air Force under Contract No. AF 61(052)—43. This grant is gratefully acknowledged.

I am indebted to Mr. S. Sandborg for help with the X-ray examination of $TaCl_5 \cdot POCl_3$.

The approximate cell dimensions are:

1. $TaCl_5 \cdot POCl_3$:	Orthorhombic,	Space group,	$P\,n\,m\,a$.
	$a = 16.4$ Å	$b = 8.1$ Å	$c = 9.0$ Å
2. $TiCl_5 \cdot 2POCl_3$:	Orthorhombic,	Space group,	$P\,n\,n\,m$.
	$a = 13.4$ Å	$b = 13.5$ Å	$c = 7.7$ Å

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Received August 6, 1962.