FINSKA KEMISTSAMFUNDETS MEDDELANDEN

SUOMEN KEMISTISEURAN TIEDONANTOJA

REDAKTÖR — TOIMITTAJA

Tor-Magnus Enari

INNEHÅLL — SISÄLTÖ

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Utgiven av — Julkaisija
Finska Kemistsamfundet — Suomen Kemistiseura
Postbox 476 Postilokero
Helsingfors — Helsinki

Styrelse — Hallitus
JARL JOHAN LINDBERG — TOR-MAGNUS ENARI — CARL ENEBÄCK — TERJE ENKVIST
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Redaktör — Toimittaja
TOR-MAGNUS ENARI, Morsviksvägen 1 Maamonlahdentie tel 55 022, 67 48 24 puh
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Preparation of β-Amylases of Barley

M. Nummi, Raili Vilhunen and T-M. Enari

Laboratory of Brewing, Helsingfors, Finland

The salting-out of barley β-amylases of different molecular size was studied. The β-amylases of larger molecular size (A_4 and A_3) were precipitated by ammonium sulphate at the concentration of 20 % and the smaller β-amylases (A_2 and A_1) between 20 and 40 % of saturation concentration.

A method has been developed for the preparation of the barley β-amylases of different molecular size. The method is based on the salting-out with ammonium sulphate and exclusion chromatography of the different β-amylases.

According to our earlier experiments, there are at least four water soluble β-amylases of different molecular size.[1] The components were separated using exclusion chromatography on

Sephadex G-100 and they were called A_1, A_2, A_3 and A_4 from the smallest to the largest. All the different β-amylases are immunochemically identical [2] and thus probably aggregates of the same fundamental component.

A method has been developed for the preparation of the different β-amylases of barley. The amylases obtained have been further analysed using ultracentrifugal [3] and immunoelectrophoretical [2] methods. The preparation includes two steps:

1) precipitation with ammonium sulphate
2) exclusion chromatography on Sephadex G-100

After the chromatography the different β-amylase fractions are still not pure but contain other albumins of the corresponding molecular size. This fact is somewhat disadvantageous in the ultracentrifugations but does not cause any trouble in the immunoelectrophoresis where the enzymatic activity of the β-amylases can be used for the detection of these proteins.

Material and Methods

In all experiments barley of the six-row variety, *Pirkka*, was used.

β-Amylase activity: The β-amylase activities were determined with a modification[1] of the method of Kirsop[4] using 3,5-dinitrosalicylic acid as reagent at pH 7.0. All the β-amylase activities are expressed as units of

$$\frac{\text{mg maltose}}{\text{ml sample} \times \text{min.}}$$

Exclusion chromatography: The exclusion chromatography on Sephadex G-100 columns was performed as described by Nummi[5]. Phosphate buffer (0.010 *M*, pH 7.5) which contained 2.5 % NaCl was used in the chromatography. All runs were made on the same column (4 × 66 cm).

Gel filtration: A Sephadex G-25 column (4 × 60 cm) was used to remove the high amounts of ammonium sulphate in the solution of A_1 and A_2. Furthermore, at the same time the traces of globulins were separated from albumins, which included the β-amylase, by using a water packed column and elution with 2.5 % NaCl solution. The albumin fractions were collected according to the drawing in the figure. The conductivity of the albumin solutions was about 100 μS/cm.

Purification on DEAE-cellulose: In the preparation of A_1 and A_2 the amylase solution obtained through gel filtration on Sephadex G-25 was purified on DEAE-cellulose using step-wise elution. The albumin solution was buffered with 0.005 *M* sodium phosphate to pH 7.5. The albumins were adsorbed on DEAE-cellulose which was equilibrated with the same buffer. The cellulose was thereafter packed to a column with the dimensions 4 × 11 cm and the basic proteins were removed by washing the column with 130 ml of a 0.005 *M* sodium phosphate solution (pH 7.5). Elution was performed with a 0.1 *M* sodium phosphate solution (pH 7.0).

Salting out: Before the precipitations with ammonium sulphate the pH of the protein solutions was adjusted to 7.5 with a sodium phosphate solution giving a final concentration of 0.010 *M* and 2.5 % of sodium chloride was added. Dry ammonium sulphate was added to these solutions to give the concentrations stated. The amounts of ammonium sulphate were calculated assuming that the saturated solution contains 760 g/l.

Table 1. Ammonium sulphate precipitation of β-amylase

ammonium sulphate % of saturation	protein in precipitate %	β-amylase in precipitate %
0—20	26.8	23.6
20—30	22.0	46.8
30—40	19.8	26.3
40—50	19.7	2.8
50—60	10.5	0.5
60—70	0.7	0.0
70—100	0.5	0.0

Extraction of β-Amylases

Free β-amylase: Acetone treated barley was extracted for two hours with distilled water (1 g/2 ml) at +4°C. After centrifugation 2.5 % of sodium chloride was added to the clear extract in order to prevent inactivation of the β-amylase. The pH of the extract was also adjusted to 7.5 with a sodium phosphate solution giving a final concentration of 0.010 M. The extract was then kept at refrigerator temperature for 2 days, during which time interfering substances were hydrolysed[7].

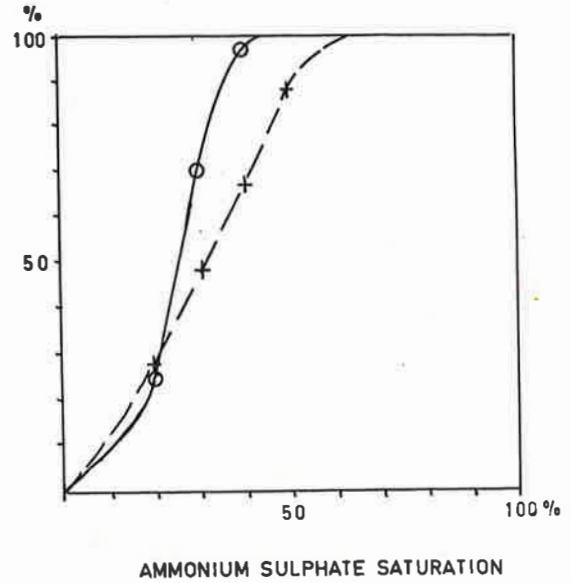

Fig. 1. Ammonium sulphate precipitation of β-amylase of barley

——·—·— = precipitated β-amylase (%)
— — — — = precipitated protein (%)

Table 2. Ammonium sulphate precipitation of β-amylase fractions.

ammonium sulphate % of saturation	β-amylase activity			
	A_1	A_2	A_3	A_4
0—20	19 %	31 %	100 %	100 %
20—30	54 %	60 %	—	—
30—40	27 %	9 %	—	—

Bound β-amylase: The albumins were first washed out with distilled water (1 g/3 ml). The washing was repeated 8 times. The globulins were also removed with an 8 time washing by using a 2.5 % sodium chloride solution, and then the salt was removed by washing 8 times with water. All the washings were performed at +4°C. The bound β-amylase was extracted (1 g/2 ml) for 4 days with a 0.3 % thioglycolic acid solution, pH 7.0.

Preliminary Experiments

In order to find the suitable ammonium sulphate concentrations for the precipitation of the β-amylases of different molecular size, a progressive precipitation with 10 % intervals was

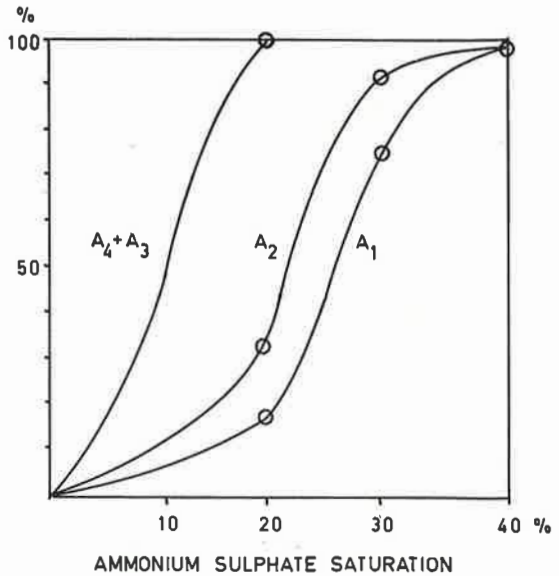

Fig. 2. Ammonium sulphate precipitation of different β-amylase fractions

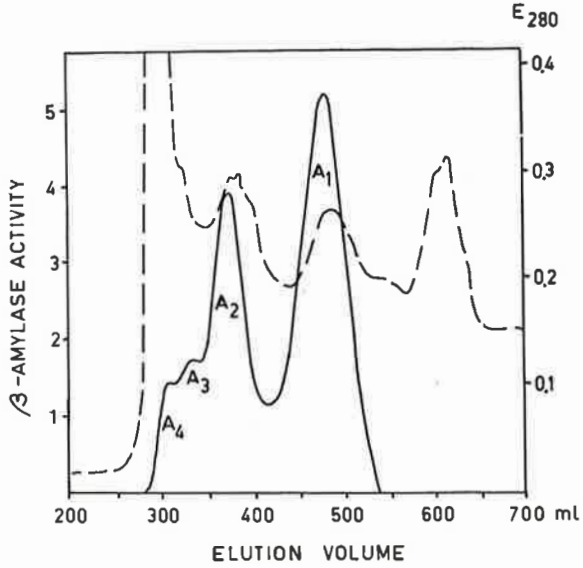

Fig. 3. Chromatography of barley β-amylase on Sephadex G-100. Column: 4 × 65 cm.

——— = β-amylase activity
— — — = protein concentration

performed. The precipitations were carried out at +4°C and the precipitation time was 2 hours. The precipitates were then washed with a corresponding ammonium suplhate solution also containing 0.010 *M* phosphate, pH 7.5, and 2.5 % sodium chloride. After the washing the precipitates were dissolved in the phosphate buffer containing 2.5 % sodium chloride. The protein concentrations and β-amylase activities of the dissolved precipitates are collected in Table 1 and in Fig. 1.

The fractions from the ammonium sulphate precipitation were then fractionated again on a Sephadex G-100 column. The results are collected in Table 2 and in Fig. 2.

The values of the activities in Table 2 are approximate since the β-amylase activities were calculated from the heights of the peaks in the exclusion chromatography.

Preparation of the Largest β-Amylase Component, A_4.

The β-amylases of larger molecular size (A_3 and A_4) are completely precipitated by 20 % saturation with ammonium sulphate as can be seen in Table 2 and Fig. 2. This percentage of saturation precipitates in addition to A_4 and A_3 appreciable

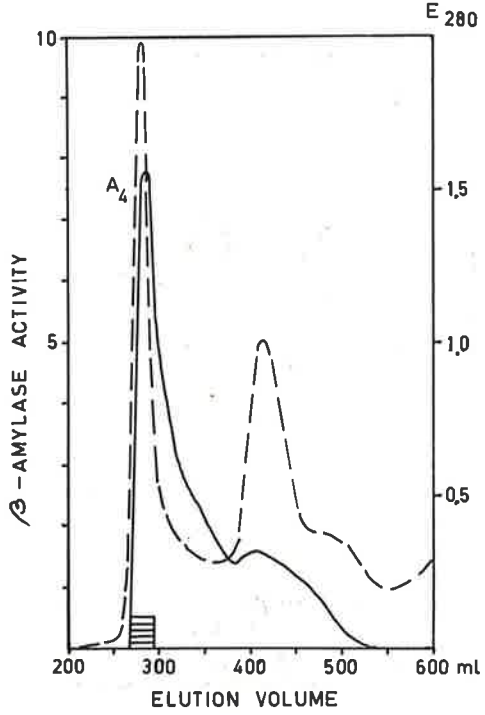

Fig. 4. Chromatography of precipitated A_4-fraction on Sephadex G-100. (Fraction obtained by precipitation with 0—18 % saturation of ammonium sulphate)

——— = β-amylase activity
— — — = protein concentration

amounts of A_2 and A_1. Therefore, 18 % saturation was chosen for the precipitation. The precipitates were washed several times with an ammonium sulphate solution to remove the smaller β-amylase components. Finally the large molecular β-amylase was purified by chromatography on Sephadex G-100. The final method for preparation of component A_4 was as follows:

1.5 kg of acetone-treated finely ground barley was extracted with water. 2.5 % of sodium chloride, phosphate buffer and ammonium sulphate were added to the obtained extract to give an 18 % saturation of ammonium sulphate; the precipitate was allowed to settle for 2 hours. The centrifuged precipitate was washed 4 times with a similar ammonium sulphate solution. The washed precipitate was dissolved in the phosphate buffer giving a final volume of 40 ml.

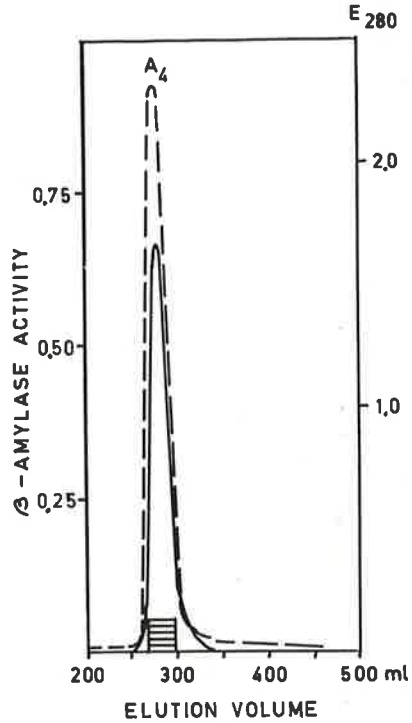

Fig. 5. Rechromatography of A_4-fraction shown in Fig. 4.

——— = β-amylase activity
— — — = protein concentration

The solution obtained was further fractionated on Sephadex G-100 in 8 ml samples. The elution curve is shown in Fig. 4. The results were highly reproducible in every run. A comparison with the elution curve of the total β-amylase of a water extract of barley (Fig. 3) shows that the largest β-amylase is enriched and purified from other β-amylase components.

In order to purify it further, rechromatography of the A_4 component was necessary. The β-amylase was collected from the previous runs according to the drawing in Fig. 4. As can be seen in Fig. 5, the A_4-amylase is eluted exactly at the same place as before and is now free from the other β-amylase components. The collected A_4-fraction (according to the drawing in Fig. 5) was desalted by gel filtration. Thereafter, the solution was freeze dried and used for immunoelectrophoretical studies [2] or dialyzed against the buffer and used for ultracentrifugal analyses[3].

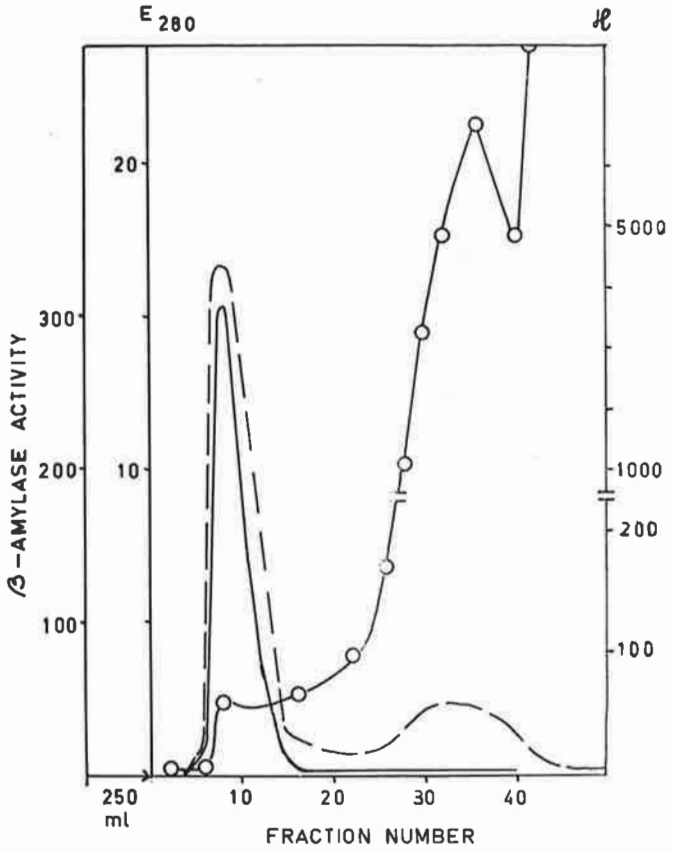

Fig. 6. Gel filtration of precipitated $A_1 + A_2$-fraction on Sephadex G-25. (Fraction obtained by precipitation between 23—30 % saturation of ammonium sulphate).
Column: 4 × 60 cm. Packed with distilled water. Elution with 0.010 M phosphate buffer (pH 7.5) containing 2.5 % NaCl. Fractions: 11 ml.

——— = β-amylase activity
— — — = protein concentration
—O—O— = conductivity (μS/cm)

Preparation of the Smaller β-Amylase Components, A_2 and A_1

The preliminary experiments showed (Fig. 2) that the components A_2 and A_1 are precipitated almost completely and in the purest state between 20 and 30 % of saturation with ammonium sulphate. To avoid precipitation of larger components the interval of precipitation was minimized to 23—30 %. After precipitation the small molecular substances, especially the high

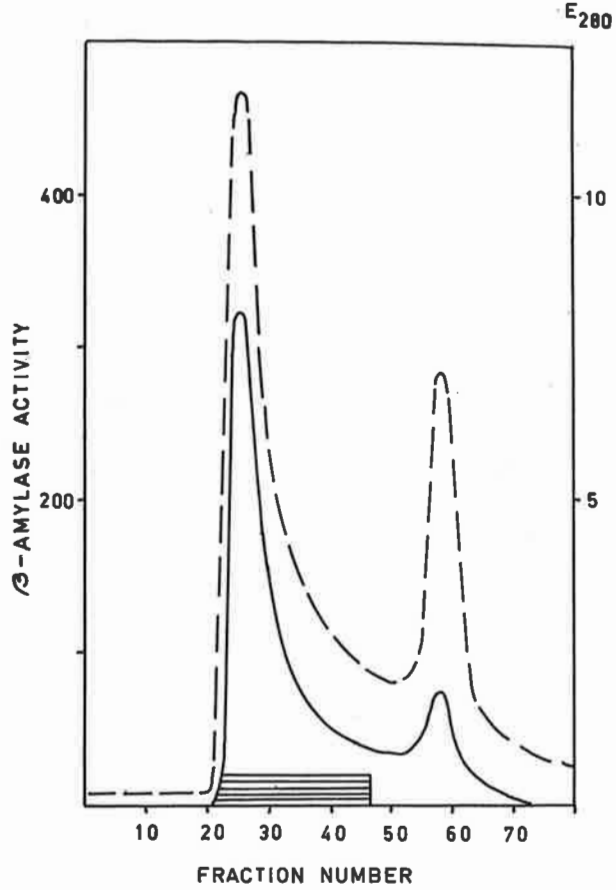

Fig. 7. Chromatography of $A_1 + A_2$-fraction on DEAF-cellulose. Column: 4 × 11 cm. Elution with 0.1 M Na-phosphate, pH 7.0. Fractions: 5 ml.

——— = β-amylase activity
—O—O— = protein concentration

amount of ammonium sulphate as well as globulins, were removed with gel filtration on Sephadex G-25 using gradient elution (Fig. 6). Thereafter, the β-amylases were further purified on DEAE-cellulose and fractionated on Sephadex G-100. The final method for preparation of components A_1 and A_2 was as follows:

3 kg of acetone-treated finely ground barley was extracted with water. The fraction precipitating with ammonium sulphate between 23 and 30 % of saturation was precipitated and the obtained precipitate washed with a buffered 30 % ammonium sulphate solution. The washed precipitate was dissolved in the

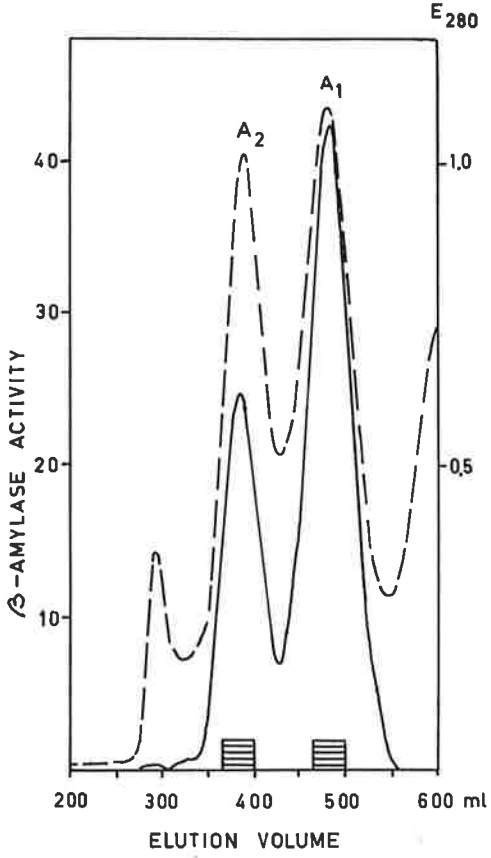

Fig. 8. Chromatography of $A_1 + A_2$-fractions on Sephadex G-100.
——— = β-amylase activity
— — — = protein concentration

0.010 *M* phosphate buffer giving a final volume of 36 ml and purified by gel filtration on Sephadex G-25 as two 18 ml samples. A typical pattern of elution is shown in Fig. 6.

The combined amylase solution was then purified from basic proteins on DEAE-cellulose. The result is shown in Fig. 7. Both the protein curve and the β-amylase activity curve show two fractions with this single elution. 80 % of the β-amylase is eluted in the first peak.

The first fraction was collected according to the drawing in in Fig. 7, and the amylases were precipitated with 40 % saturation with ammonium sulphate. The precipitate was dissolved in 10 ml of phosphate buffer and fractionated on Sephadex

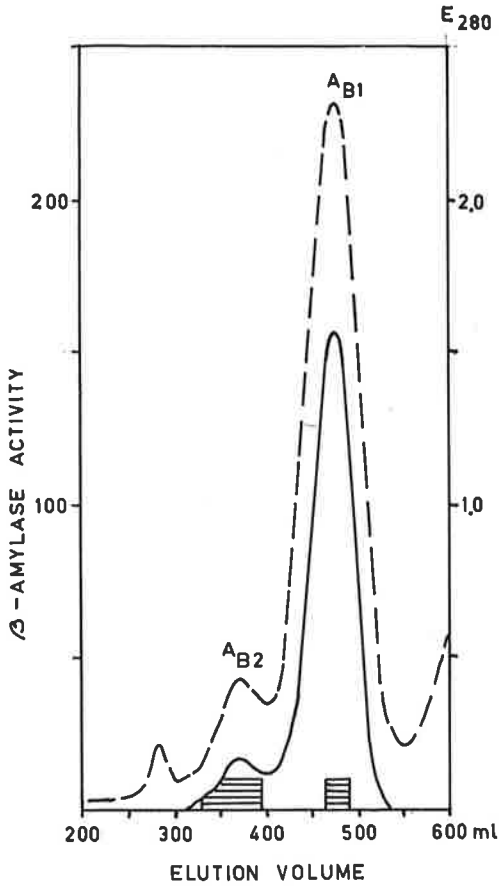

Fig. 9. Chromatography of latent β-amylases of barley on Sephadex G-100.
——— = β-amylase activity
— — — = protein concentration

G-100 as two 6 ml samples. The reproducible result is shown in Fig. 8. The fractions A_2 and A_1 are resolved fairly well and are completely free from larger β-amylases.

The collected A_1 and A_2 fractions were desalted by gel filtration and freeze dried or dialysed against the buffer and used for ultracentrifugal studies.

Preparation of the Latent β-Amylases, A_{B1} and A_{B2}

The extracted latent β-amylase was concentrated by precipitation with ammonium sulphate. The degree of saturation

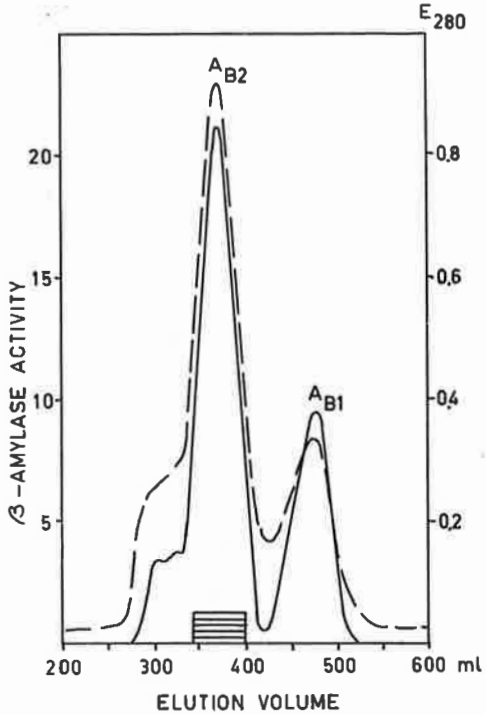

Fig. 10. Rechromatography of bound β-amylase fraction A_{B2} collected from the previous run, shown in Fig. 9.

——— = β-amylase activity
— — — = protein concentration

used for this purpose was 60 %. The precipitate obtained was dissolved in 25 ml of phosphate buffer and subjected in 4 ml samples to exclusion chromatography on Sephadex G-100. The result of the run is shown in Fig. 9. The latent β-amylase is eluted as two components, the amount of the smaller component being 90 % of the whole activity.

The smaller component A_{BI} was collected from six runs according to the drawing in Fig. 9, precipitated at 40 % of the saturation concentration of ammonium sulphate, dissolved and further treated as the previous β-amylase preparations.

In order to prepare the larger latent β-amylase for ultracentrifugations the small amounts of A_{B2}-component were collected from several runs and the combined, concentrated sample was subjected to exclusion chromatography on the same column. The result of the rechromatography is shown in Fig. 10.

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Chromatography of Phenolic Compounds on Paper Impregnated with Formamide

By *Terje Enkvist*

Experiments by Ulla Enkvist

R_f values are given for phenolic degradation products of lignin and some related substances for chromatography on paper impregnated with formamide, using two eluent systems and two different diazoreagents for spraying.

In connection with studies of lignin degradation products (1,2,3) sets of R values of phenolic substances and some alicyclic enols were needed for comparison. The present paper gives such values in the tables 1—4. The determinations were made using the descending technique on strips of about 58 × 22 cm of Whatman No 1 chromatographic paper impregnated with formamide, in the way described earlier (3). The solvent front in general moved about 40 cm. During the present work, two eluting solvent mixtures were used after saturation by shaking with formamide, namely a) methylethylketone (MEK)-xylene (5), and b) chloroform (2). These systems are rather swift (about 2 ½ hours) and effective, generally giving at least as good separation as the best systems used for instance by Reio (4). In difficult cases special systems can be used for further differentiation, as the ligroin-xylene eluent mentioned in (2), molybdate impregnated paper according to Halmekoski (6), or some of the systems of Reio. Also thin layer chromatography can be useful. Some results from it will be reported later. The chloroform originally contained as usual 1 % ethanol. Most of the alcohol was probably washed out upon shaking with formamide (no ethanol was especially added). The spraying reagents used were: a) diazotized p-nitroaniline in sodaalkaline solution (pNA), and b) diazotized sulfanilic acid(DSA). The preparation of these reagents is reported in (3). The colors are given as numbers referring to the color scale of Krieger (7); the numbers and colorations are given in table 5. The spots were inspected before spraying in ultraviolet light of wave length at about 366 mμ before and after treatment with ammonia vapor.

The absolute values of R_f can vary considerably during different determinations (cf. 5). Hence, R_f values are also given in relation to the corresponding values for pyrocatechol

(R_f pyr.) or of vanillic acid (R_f van.) These substances were always run for comparison on the same strip of paper as the other substances. For pyrocatechol absolute R_f values were obtained varying between 0.25—0.34 in methyl-ethyl-ketone(MEK)-xylene and 0.046—0.077 in chloroform.

The phenol carboxylic acids related in table 4 show very low R_f values. In spite of this, rather good differentiating results can be obtained for these, too, by allowing the chromatograms to run over the border over night and comparison with for instance vanillic acid as standard.

Discussion of results

Already in (3) some general observations concerning color and structure were reported. In addition to them the following can be mentioned: Simple monovalent phenols, such as phenol and the cresols, show colors which change with rising length of the side chain from reddish to violet with diazotized p-nitroaniline, and from yellow through orange to pale red with diazotized sulfanilic acid. Rf values with xylene — methyl ethyl ketone are between 0.79—0.89 for the phenol homologues mentioned, in chloroform about 90 % of these values. Phenol itself shows clearly lower values in both solvents.

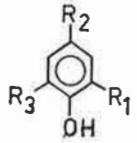
Bivalent phenols, such as pyrocatechol and its homologues show lower R_f values than the monovalent phenols, especially in chloroform. The homologues of pyrocatechol differentiate very nicely with chloroform as eluent. Trivalent phenols (pyrogallol, phloroglucinol) move still slower than the bivalent phenols.

Alkyl substituents both in the benzene nuclei and on the side chains as well as methyl in the methoxyl groups raise the R_f value especially in chloroform, so that it can become higher than the corresponding value for methylethylketone-xylene. Phenolic hydroxyl groups and methylol groups have the opposite effect. The effect of carbonyl in the side chain is weaker than that of hydroxyl. Substances with possibilities of chelation between a phenolic hydroxyl and an aldehyde group in ortho-position, such as o-vanillin, homosalicylaldehyde and compounds XXXV, XXXVI, XXXVIII, give high R_f-values in both solvent systems, and yellowish colors.

Phenols with carbonyl in conjugated position to the benzene nucleus give strong blue or violet colors in ultraviolet light with ammonia (e.g. XXIX—XXXI, 4-hydroxybenzaldehyde). An ethylene bond conjugated with the benzene nucleus seems to give weaker greenish or grey colors in ultraviolet light, especially with ammonia (isoeugenol, coniferyl alcohol, VI and VII); the stilbene derivatives pinosylvin and its monomethyl

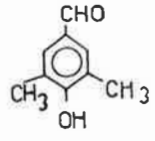
ether give radiant violet. Some of the aldehyde preparates (IV, XXXVIII) seem to give two spots. Carbonyl groups in the side chain weaken the diazo colors so much that some of these compounds cannot be detected with diazo reagents at all, for instance substances II, III, XXXIV, XLIV. These substances can be detected in ultraviolet light with ammonia, or by spraying with 2.4-dinitro-phenylhydrazine hydrochloride solution. Resorcinol and its derivatives give typical brownish yellow colors with diazotized p-nitroaniline. The colors for pyrocatechol and its derivatives are unstable, changing to grey or brown. These substances are very easily detected with the silver nitrate-ammonia (Tollens) reagent.

Syringyl type compounds (2.6 dimethoxyphenol, XX, LIV) in general give blue or bluish colors with diazotized p-nitroaniline. Bluish violet colors with diazotized p-nitroaniline and more or less red colors with diazotized sulfanilic acid are given by substances with a CH_3- or CH_2-C group bound to the benzene nucleus (for instance XII, XVII, XIX, eugenol, XXIV, XXVI). Guaiacol and its simple derivatives (XV and XVI) show violet colors with pNA, and orange with DSA. In general, alkyl groups, also methyl in methoxyl groups, seem to change the diazo colors in the direction yellow→red→violet→blue. The alicyclic enols investigated show yellow colors with the diazoreagents and higher Rf values with chloroform than with xylene-methyl-ethyl ketone. The phenol carboxylic acids (table 4) show low Rf values and mostly pale diazo colors.



	R_1	R_2	R_3
I	CH_3	CH_2OH	CH_2OH
III	CH_3	$COCH_3$	CH_3
IV	H	H	CH_2CHO
V	CH_3	CHO	CHO
VI	OH	H	$CH_2.CH = CH_2$
VII	OH	$CH_2.CH = CH_2$	H
VIII	OH	$COCH_3$	H
IX	OH	COC_2H_5	H
XII	OCH_3	CH_3	H
XIII	OCH_3	H	$CH_2CH = CH_2$
XIV	OCH_3	$CH_2CH = CH_2$	$CH_2CH = CH_2$
XV	OCH_3	CH_2OH	H
XVI	OCH_3	$CHOH.CH_3$	H
XVII	OCH_3	CH_2CH_2OH	H
XVIII	OCH_3	$CHOH.C_2H_5$	H
XIX	OCH_3	$CH_2.CH_2.CH_2OH$	H
XX	OCH_3	CH_2OH	OCH_3
XXI	OCH_3	$CH.CH.CH_2OH$	H
XXIV	OCH_3	C_2H_5	OH
XXVII	OCH_3	CH_2OH	CH_2OH

	R_1	R_2	R_3
XXVIII	OCH_3	CH_3	CH_2OH
XXIX	OCH_3	CHO	H
XXX	OCH_3	$COCH_3$	H
XXXI	OCH_3	COC_2H_5	H
XXXII	OCH_3	CH_2COCH_3	H
XXXIII	OCH_3	H	CHO
XXXIV	OCH_3	CHO	Br
XXXV	OCH_3	CH_3	CHO
XXXVI	OCH_3	C_3H_7(n)	CHO
XXXVII	OCH_3	$CH_2.COCH_2OH$	H
XXXVIII	OCH_3	CHO	CHO
XL	OCH_3	CH_3	$COCH_3$
XLVIII	OH	COOH	H
XLIX	OH	CH_2COOH	H
L	OH	CH_2CH_2COOH	H
LI	OCH_3	CH_2COOH	H
LII	OCH_3	CH_2CH_2COOH	H
LIII	OCH_3	H	COOH
LIV	OCH_3	COOH	OCH_3



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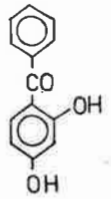
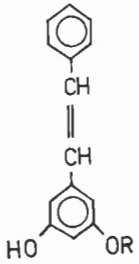
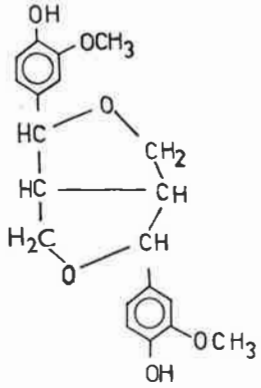


X



XI = R = H

XXII = R = CH_3



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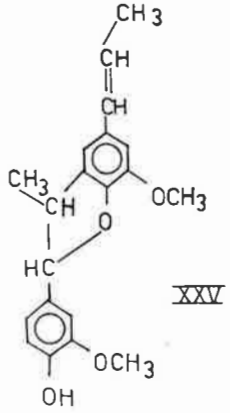
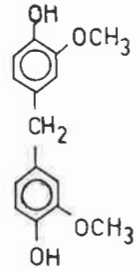


XXV



XXVI

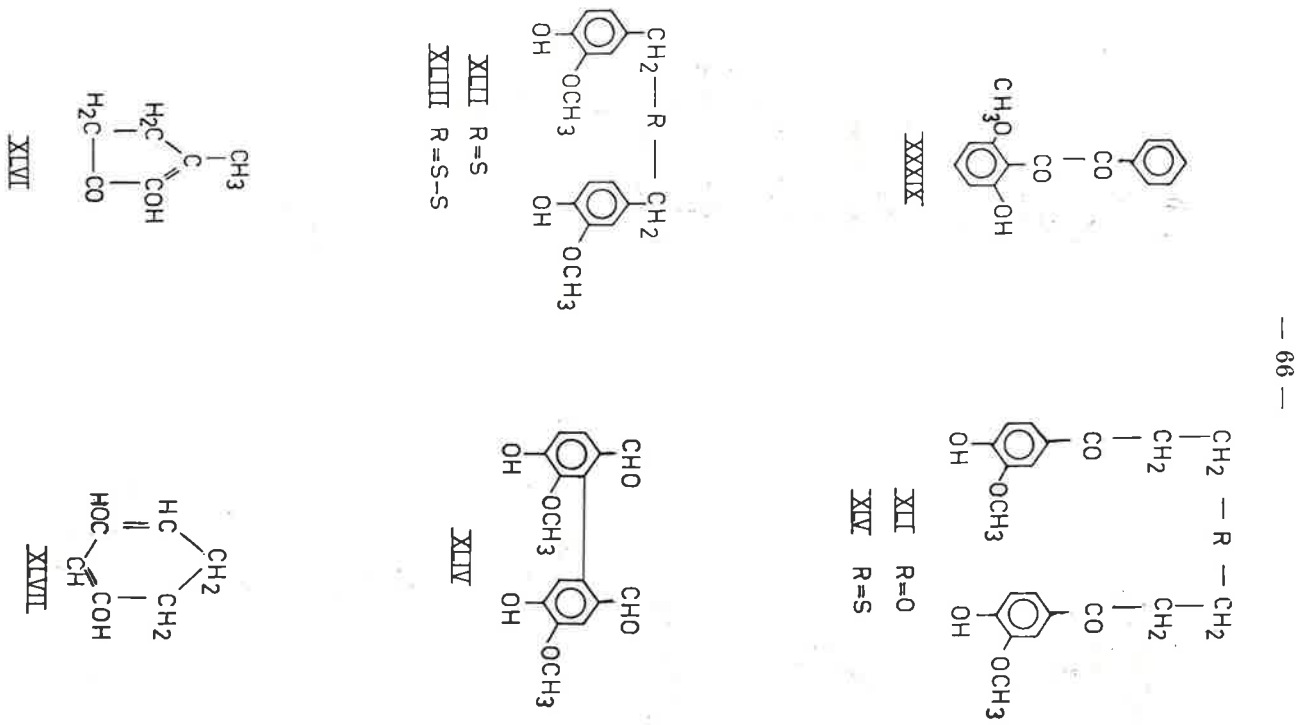


Table 1. Methoxyl free phenols.

Eluent			Xylene-MEK		Chloroform Coloration			
Substance	Rf	Rfpyr.	Rf	Rfpyr.	pNA	DSA	UV	UV—NH_3
Phenol	0.71	2.29	0.53	10.1	19	115	—	—
p-cresol	0.79	2.55	0.70	13.5	44	121	—	—
o-cresol	0.84	2.71	0.75	14.4	29	119	—	—
m-cresol	0.78	2.52	0.71	13.6	37	119	—	—
4,6-dimethylol o-cresol (I)	0.15	0.50	0.039	0.71	42	118	—	—
p-ethylphenol	0.84	3.50	0.75	13.6	37	118	—	—
p-propylphenol	0.89	2.87	0.86	16.5	42	8	—	—
4-hydroxybenzaldehyde	0.47	1.57	0.26	4.60	6 weak	122	—	Dark brown violet
4-hydroxi-3,5-dimethylbenzaldehyde (II)	0.76	2.53	0.77	14.8	—	—	—	Dark violet
4-hydroxy-3,5-dimethylacetophenone (III)	0.75	2.38	0.85	15.1	—	—	—	Radiant violet
Homosalicylaldehyde (IV)	0.58	1.87	0.78	15.0	125	5 [1]	—	Yellow
	0.90	2.90	(0.95)	(18.3)				
4-hydroxy-3-methyl-5-formyl-benzaldehyde (V)	0.84	3.00	0.93	19.4	weak	—	light yellow	radiant yellow
Pyrocatechol	0.30	1.00	0.052	1.00	102	123→100	—	—
4-methylcatechol	0.35	1.25	0.10	1.92	49	7	—	—
4-ethylcatechol	0.50	1.79	0.16	3.15	42	118	—	—
4-propylcatechol	0.58	2.07	0.27	4.91	33	8	—	—
2-allylcatechol (VI)	0.59	2.18	0.37	7.00	77	77	light gray	—
4-allylcatechol (VII)	0.49	1.82	0.22	4.15	33	145	» »	—
Resorcinol	0.22	0.68	0.019	0.35	115	112	—	—
Pinosylvin (XI)	0.67	2.16	0.11	2.11	124	112	radiant violet	radiant violet
Pyrogallol	0.05	0.17	0.007	0.18	128	129	—	—
Protocatechualdehyde	0.18	0.60	0.023	0.48	111	111	—	Dark violet
4-acetocatechol (VIII)	0.14	0.50	0.033	0.69	131	131	—	Radiant violet
4-propiocatechol(IX)	0.25	0.90	0.115	1.59	124 [2]	124 [2]	—	—
2.4-dihydroxybenzophenone (X)	0.90	2.81	0.82	14.6	132	128	Dark violet	Dark violet
Hydroquinone	0.18	0.55	0.012	0.18	145	145	—	—
Phloroglucinol	0.044	0.13	0	0	115	115	—	—

[1]) Red spot(color 5) with violet border (color 41). [2]) Pale

Table 2. Methoxyl containing phenols. Hydrocarbon or alcohol side chains.

Eluent	Xylene-MEK		Chloroform		COLORATION			
Substance	Rf	Rpyr.	Rf	Rpyr.	pNA	DSA	UV	UV-NH_3
Guaiacol	0.71	2.23	0.90	14.8	42	119	—	—
Creosol (XII)	0.80	2.64	0.90	15.1	45	19	—	—
4-ethylguaiacol	0.84	2.55	0.90	15.1	52	19	—	rad. blue
4-propylguaiacol	0.87	2.75	0.92	16.7	42	19	—	light violet
Eugenol	0.86	2.87	0.92	17.7	45	19	—	—
Isoeugenol	0.87	3.22	0.93	16.6	93	17	—	—
o-eugenol (XIII)	0.92	2.63	0.95	15.6	45	119	—	Greenish
o-allyleugenol (XIV)	0.85	2.58	0.93	17.9	45	19	—	—
2.6-dimethoxyphenol	0.57	1.96	0.91	21.8	59	9		
Vanillylalcohol (XV)	0.13	0.48	0.19	4.3	42	119	—	—
Apocynol (XVI)	0.21	0.70	0.36	6.90	42	119	—	—
2-guaiacylethanol (XVII)	0.20	0.59	0.35	6.15	45	37	—	Light violet
1-guaiacylpropanol (XVIII)	0.32	1.25	0.63	11.3	44	120	—	—
3-guaiacylpropanol (XIX)	0.25	1.04	0.45	8.6	45	19→26	—	—
Syringylalcohol (XX)	0.069	0.25	0.27	6.1	59	10	—	—
Coniferylalcohol (XXI)	0.44	1.63	0.76	13.6	99	10	light	yellow green
Pinosylvin monomethylether (XXII)	0.85	2.74	0.90	17.3	19	124	rad. violet	radiant violet
Pinoresinol (XXIII)	0.33	0.94	0.90	14.8	44	19	—	—
6-hydroxy-4-ethylguaiacol (XXIV)	0.46	1.53	0.68	1.48	44	6	—	—
Dehydrodiiseugenol (XXV)	0.93	3.44	0.95	17.0	44	17	—	—
Diguaiacylmethane (XXVI)	0.54	2.00	0.86	15.4	44	138	—	—
4.6-dimethylolguaiacol (XXVII)	0.033	0.12	0.023	0.52	33	119	—	—
o-methylol-4-methylguaiacol (XXVIII)	0.31	1.07	0.61	11.7	40	19	—	—

Table 3. Methoxyl and carbonyl or sulfur conatining phenols-Alicyclic enols

Eluent	xylene-MEK		Chloroform		Coloration			
Substance	Rf	RFpyr.	Rf	Rfpyr.	pNA	DSA	UV	UV−NH_3
Vanillin (XXIX)	0.50	1.79	0.72	15.0	42 [1])	125 [1])	—	Dark violet
Acetovanillone (XXX)	0.45	1.50	0.80	15.4	32	6	—	Radiant
Propiovanillone (XXXI)	0.62	2.20	0.87	19.8	42	6	—	Radiant violet
Guaiacylacetone (XXXII)	0.47	1.88	0.65	11.8	—	—	—	violet
o-vanillin (XXXIII)	0.75	2.68	0.92	12.8	37	125	white	yellow
5-bromovanillin (XXXIV)	0.62	2.07	0.73	14.0	—	—	—	dark violet
4-methyl-6-formylguaiacol (XXXV)	0.82	2.73	0.96	18.5	112	114	yellow	Bright yellow
4-propyl-6-formylguaiacol (XXXVI)	0.89	2.81	0.96	17.1	125	125	light yellow green	light yellow green
1-guaiacyl-3-hydroxyacetone (XXXVII)	0.86	2.69	0.25	4.47	34	19	light	light
Guaiacoldialdehyde (4-hydroxy-5-methoxyisophtal-	(0.78)	(3.12)	(0.63)	(12.1)	—	—	radiant	
aldehyde (XXXVIII))	0.25	1.00	0.95	18.3			yellow green	yellow green
o-hydroxy-o-methoxybenzil (XXXIX)	0.84	3.00	0.94	13.1	7 weak	125	light	light
4-methyl-6-acetovanillone (XL)	0.87	2.80	0.95	20.7	34	111	light	light
2.2′-divanilloyl-diethylether (XLI)	0.35	1.09	0.79	14.1	37	5	—	Radiant violet
Vanillylmonosulfide (XLII)	0.66	3.47	0.86	16.5	49	19	—	—
Vanillyldisulfide (XLIII)	0.79	2.63	0.92	20.0	44	37	—	—
Dehydrodivanillin (XLIV)	0.77	2.57	0.95	14.2	—	—	—	radiant
Vanilloyldiethylsulfide(XLV)	0.51	1.70	0.85	16.3	32	6	—	Radiant violet
Methylcyclopentenolone (XLVI)	0.40	1.29	0.68	13.1	111	111	—	—
Dihydroresorcinol (XLVII)	0.12	0.41	0.40	5.26	117→ 49	122	—	—
Cyclohexanedione 1,2	0.55	1.90	0.87	11.4	117→101	124	—	—

[1]) Weak

Table 4. Phenol carboxylic acids.

Eluent	Xylene-MEK		Chloroform			Coloration		
Acid:	Rf	Rfvan [1])	Rf	Rfvan [1])	pNA	DSA	UV	UV−NH_3
Vanillic	0.12	1.00	0.059	0.98 [2])	42	119	—	—
Salicylic	0.14	1.17	0.024	0.41	123 [3])	—		
m-hydroxibenzoic	0.09	0.75	0.009	0.15	10	115	—	—
p-hydroxibenzoic	0.15	1.28	0.013	0.22	131	112	—	—
Protocatechuic (XLVIII)	0.038	0.32	0.005	0.079	41	7	—	—
Homoprotocatechuic (XLIX)	0.024	0.20	0	0	41	115	—	—
Dihydrocaffeic (L)	0.044	0.37	0.006	0.10	41	7	white	violet
2.4-dihydroxybenzoic	0.028	0.23	0.004	0.062	140	128	—	—
3.5- »	0.014	0.24	0.003	0.058	126	125	—	—
2.5- »	0.033	0.28	0.008	0.14	130	130	—	—
Homovanillic (LI)	0.061	0.51	0.045	0.77	45	7	—	—
3-guaiacylpropionic (LII)	0.14	1.18	0.14	2.39	45	25	—	—
o-vanillic (LIII)	0.030	0.25	0.011	0.19	123	19	light blue	blue violet
Isovanillic	0.096	0.80	0.043	0.73	42	7	—	—
Syringic (LIV)	0.056	0.47	0.090	1.52	52	8	—	—
Gallic	0.022	0.18	0.003	0.05	145	145	dark	light

[1]) Rf value compared with that of vanillic acid
[2]) In this case only: Rf value compared with that of pyrocatechol.
[3]) Weak

Table 5. Coloration

No	
5	Vermilion (red)
6	pale salmon
7	pale orange red
8	pale red
9	brownish red
10	brown red
17	pink
19	carmine
25	lilac rose
26	rose lilac
29	lilac
32	brownish lilac
33	brown lilac
34	grey lilac
37	pale purple
40	purple-violet
41	pale violet
42	bright violet
44	bluish violet
45	blue violet
49	grey violet
52	pale violet blue
56	violet ultramarine
59	ultramarine
77	dark blue green
93	olive green
99	yellow-olive
100	brown olive
101	olive grey
102	lilac-grey
111	dull yellow
112	greenish yellow
114	sulfur yellow
115	yellow
117	dark chrome
118	orange yellow
119	orange yellow
120	orange
121	red orange
122	cream
123	buff
124	straw yellow
125	olive yellow
126	ochre
128	ochre brown
129	olive brown
130	pale brown
131	yellow brown
132	orange brown
138	carmine brown
140	lilac brown
145	grey brown

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Notiser — Uutisia

Suomalaisten Kemistien Seura's 11. fortbildningskurs hålls 10—12. 1. 1966 på Helsingfors Universitets Biokemiska institution. Kurstema är elektrokemins analytiska tillämpningar och avsikten är att ge kemister tillfälle att bekanta sig med de möjligheter de nyaste elektrokemiska analysmetoderna erbjuder. I samband med kursen anordnas demonstrationer samt en utställning av laboratorieapparatur i Forsthuset 11—12. 1. 1966.

Som kursföreläsare fungerar prof. N. Ellfolk (preparativ elektrofores), fil.mag. J. Harri (specialelektroder), fil.dr. J. J. Lindberg (organisk polarografi), dipl. ing. M. Manninen (kantvågspolarografi), prof. A. Meretoja (den potentiometriska titreringens grunder), fil.mag. S. Närvänen (högspänningselektrofores), fil.dr. N—E Saris (immunoelektrofores), tekn.lic. G. Sundholm (nya polarografiska analysmetoder) och tekn.dr. E. Wänninen (komplextitreringens potentiometri).

Anmälningar till kursen emottagas på Suomalaisten Kemistien Seura's kansli Skillnadsg. 3, Helsingfors.

Deltagaravgifter: Suomalaisten Kemistien Seura's medlemmar 100:—, icke-medlemmar 150:—. Deltagare från forsknings- eller undervisningsanstalter beviljas 40:— rabatt.

XXXVIth International Congress on Industrial Chemistry

The XXXVIth International Congress on Industrial Chemistry, under the High Patronage of H. M. King Baudouin, and traditionally placed under the auspices of the »Société de Chimie Industrielle», having its head office in Paris, will hold its sittings in Brussels from 10th to 21st September 1966.

As was the case for the 1958 Congress which took place in Liège, this important event will be organized in conjunction with the »Fédération des Industries Chimiques de Belgique» and the Belgian Branch of the »Société de Chimie Industrielle».

It can be forecasted that this meeting, which will be characterized by a series of lectures delivered by scientists of high repute and where hundreds of papers on topics relating to the chemical industry and allied branches will be delivered, will, as on previous occasions, attract a large number of people.

Hereunder is detailed the scientific programme:

I. *Organization of research*
II. *General technical problems of the chemical industry*
III. *Fuels*
IV. *Nuclear sciences*
V. *Metallurgy*
VI. *Mineral Chemicals Industries*
VII. *Silicates Industry*
VIII. *Petrochemistry and Coalchemistry*
IX. *Photographic, pharmaceutical and dyestuffs industries*
X. *Miscellaneous organic industries*
XI. *Food and agricultural industries*
XII. *Industrial and social organization*

The intention of presenting a paper must be notified *before 1st December 1965*
Typewritten papers will not be accepted *after 1st April 1966*

All further information may be obtained at the:

General Secretariat of the
XXXVIth INTERNATIONAL CONGRESS ON INDUSTRIAL CHEMISTRY
49, Square Marie-Louise,
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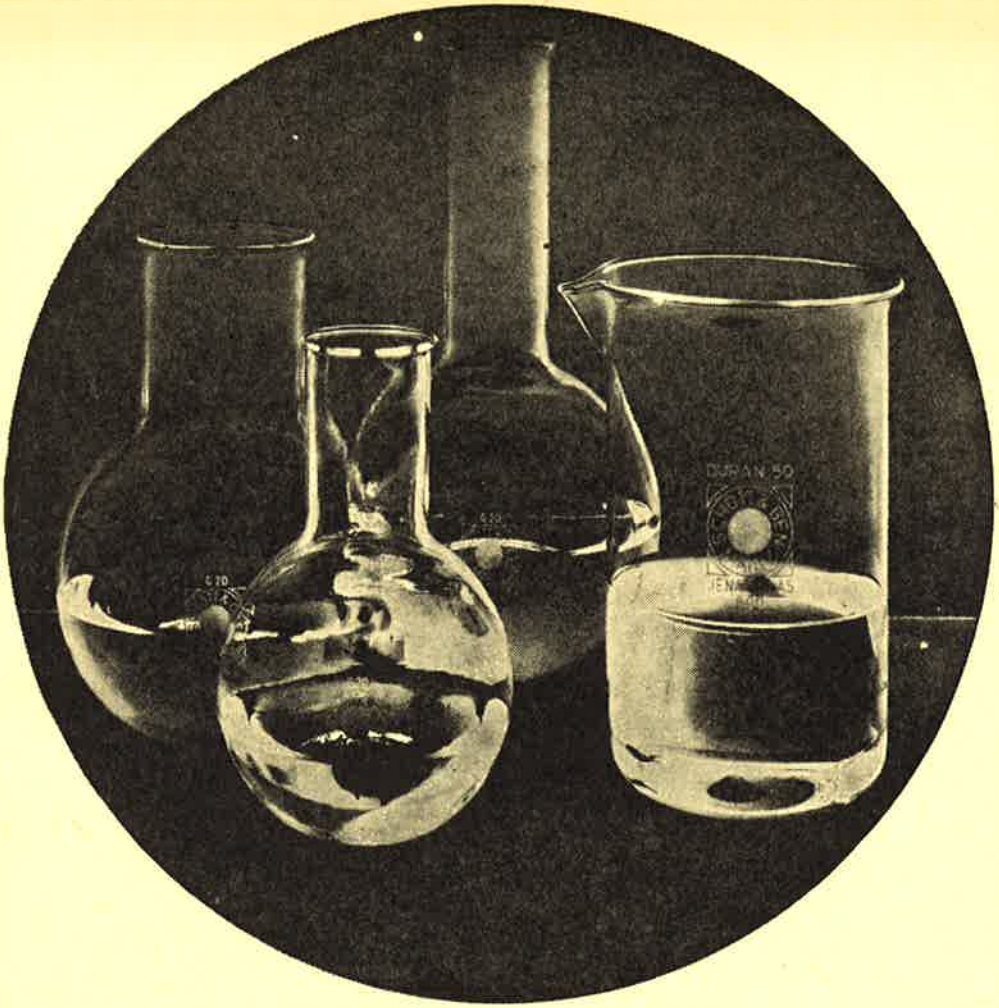

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