the starting material is the pure derivative of D-configuration.

Experimental

Carboallyloxyamino Acids.—The carboallyloxy amino acids were prepared as described in a previous article.[8]

Papain.—Commercial papain (Merck) was purified by the general procedure of Grassmann,[12] and Bergmann and Fraenkel-Conrat.[4] After two successive treatments with hydrogen sulfide, followed by precipitation with methanol, the preparation was lyophilized, yielding a light powder.

Enzymatic Syntheses.—The carboallyloxyamino acid phenylhydrazides were prepared by incubating carboallyloxyamino acids with phenylhydrazine in a buffered solution of papain and cysteine hydrochloride at 40° according to the method of Bergmann and Fraenkel-Conrat.[4] Certain of the results are listed in Table I.

Comparison of Enzymatic Synthesis from Derivatives of L-Leucine and DL-Leucine.—Two parallel runs were made. In one experiment, 0.2 mole (43 g.) of N-carboallyloxy-DL-leucine was incubated with 10 ml. of phenylhydrazine, 4 g. of L-cysteine hydrochloride, and 2 g. of papain in 1 l. of solution buffered with acetic acid–sodium acetate to *p*H 4.8. In the other experiment, 0.1 mole (21.5 g.) of N-carboallyloxy-L-leucine was incubated with 10 ml. of phenylhydrazine, 2 g. of L-cysteine hydrochloride, and 1 g. of papain in 500 ml. of solution, buffered with acetic acid–sodium acetate at *p*H 4.8. At intervals, the solid product was collected, and the filtrates incubated further. The melting point and specific rotation was taken for each sample of precipitate. The results are shown in Table II.

N-Carboallyloxy-D-leucine.—After the mixture of N-carboallyloxy-DL-leucine, papain and phenylhydrazine described above had incubated for 144 hours, 23.6 g. of N-carboallyloxyleucine of predominantly the L-form had been removed from the solution. The solution containing unreacted N-carboallyloxy-D-leucine was evaporated to dryness. The resulting solid was dissolved in 100 ml. of 6 *N* hydrochloric acid and heated under reflux for three hours. This solution was then evaporated to dryness, the residue dissolved in 50 ml. of water, and concd. ammonium hydroxide added to *p*H 6. After cooling, 0.8 g. of crystals was collected. When recrystallized from alcohol–water, the yield of D-leucine was 0.55 g., $[\alpha]^{20}D$ −14.8° (3.6% in 20% hydrochloric acid). The D-leucine was converted to N-carboallyloxy-D-leucine by the usual method.

N-Carboallyloxy-D-leucine Phenylhydrazide.—A solution consisting of 0.2 g. of N-carboallyloxy-D-leucine, 0.067 g. of L-cysteine hydrochloride, 0.05 g. of purified papain, and 0.5 ml. of phenylhydrazine in 15 ml. of solution buffered to *p*H 4.8 was incubated at 40°. After twelve days the insoluble product was collected, yielding 0.177 g. of N-carboallyloxy-D-leucine phenylhydrazide; m. p. 110°. Recrystallized from toluene, it melted at 111–112°. When mixed with an equal amount of N-carboallyloxy-L-leucine phenylhydrazide, the melting point was 153–154°.

(12) Grassmann, *Biochem. Z.*, **279**, 131 (1935).

Summary

From the treatment of a series of N-carboallyloxy-derivatives of α-amino acids with phenylhydrazine in the presence of papain, the crystalline phenyl hydrazides have been obtained in every case. While the carboallyloxy derivatives of L-amino acids yield the corresponding L-phenylhydrazides the derivatives of DL-amino acids do not invariably yield the optically pure L-phenylhydrazide. Under the conditions of the experiments, the D-phenylhydrazide is simultaneously formed to an appreciable though much smaller extent.

PULLMAN, WASHINGTON RECEIVED AUGUST 2, 1949

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Reactions of Vanillin and its Derived Compounds. IX.[1] Some New Esters of Syringic and Protocatechuic Acids[2]

BY IRWIN A. PEARL AND DONALD L. BEYER

The possible utilization of the lignin and/or the waste liquors from the sulfite pulping of coniferous woods by oxidation to vanillin and transformation of the resulting vanillin to vanillic acid has been reviewed[3] and the direct oxidation of lignosulfonates to vanillic acid has been reported recently.[4] Ester of vanillic acid and of relateds acids derived from vanillin have been prepared and tested for their toxicity toward representative microörganisms and their ultraviolet absorption properties.[5]

Oxidations of lignins and sulfite waste liquors derived from the pulping of hardwoods by processes similar to those employed with softwood lignins yield considerable amounts of syringaldehyde and syringic acid in addition to vanillin and vanillic acid. This fact, together with the well-known fact that more and more hardwoods are being processed by our sulfite pulp mills, led to an investigation of the esters of syringic acid.

Syringic acid for this study was prepared by treatment of trimethylgallic acid with concentrated sulfuric acid or with fuming sulfuric acid by the method of Bogert and Coyne[6] and Bogert and Ehrlich,[7] respectively. When employing the concentrated sulfuric acid method, we found it impossible to obtain syringic acid free from trimethylgallic acid unless the trimethylgallic acid was recrystallized before the sulfuric acid treatment. Such recrystallization before treatment obviated the necessity for purification of the syringic acid which could be employed as such for the esterifi-

(1) For paper VIII of this series, see THIS JOURNAL, **71**, 2331 (1949).

(2) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results.

(3) Pearl, *Chem. Eng. News*, **26**, 2952 (1948).

(4) Pearl, THIS JOURNAL, **71**, 2196 (1949).

(5) (a) Pearl and McCoy, *ibid.*, **69**, 3071 (1947); (b) Pearl and Beyer, *ibid.*, **71**, 1066 (1949); (c) Pearl, *ibid.*, **71**, 2331 (1949).

(6) Bogert and Coyne, *ibid.*, **51**, 571 (1929).

(7) Bogert and Ehrlich, *ibid.*, **41**, 799 (1919).

TABLE I

ESTERS OF PROTOCATECHUIC ACID

No.	Ester[a]	Yield, %	M. p.,[b] °C.	Solv.[c]	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Inhibiting concentrations[d] *Aerobacter aerogenes*	*Bacillus mycoides*	*Aspergillus niger*
1	Me[e,f]	60	133	A						0.210	0.090	>0.210
2	Et[f]	77	130	B						.150	.090	> .210
3	iso-Pr	42	132	C	$C_{10}H_{12}O_4$	61.22	61.03	6.16	6.18	.090	.090	> .210
4	Pr[f]	85	114	C						.090	.090	> .210
5	iso-Bu	87	119	C	$C_{11}H_{14}O_4$	62.84	62.97	6.71	6.77	.030	.015	.150
6	Bu	86	115	C	$C_{11}H_{14}O_4$	62.84	62.77	6.71	6.76	.021	.015	.090
7	iso-Am	96	109–110	C	$C_{12}H_{16}O_4$	64.27	64.26	7.19	7.19	.015	.009	.090
8	Am	76	94	C	$C_{12}H_{16}O_4$	64.27	64.13	7.19	7.17	.015	.009	.030
9	Et_2CHCH_2	87	79	C	$C_{13}H_{18}O_4$	65.53	65.34	7.61	7.59	.090	.003	> .210
10	Ph[g]	47	190	D						.030	.009	.150
11	$MeOCH_2CH_2$	67	146	E	$C_{10}H_{12}O_5$	56.60	56.60	5.70	5.80	.210	.210	> .210
12	$PhOCH_2CH_2$[e]	71	136	F	$C_{15}H_{14}O_5$	65.68	65.35	5.14	5.17	.021	.009	> .210
13	$ClCH_2CH_2$[e]	96	132	D	$C_9H_9O_4Cl$	49.90	50.02	4.19	4.21	.090	.090	> .210
14	$MeCHClCH_2$[e]	77	112	D	$C_{10}H_{11}O_4Cl$	52.07	52.17	4.81	4.85	.090	.090	> .210
15	$ClCH_2CHClCH_2$[e]	93	118	D	$C_{10}H_{10}O_4Cl_2$	45.31	45.36	3.80	3.80	.030	.015	> .210
16	$(CH_2Cl)_2CH$	66	90–91	D	$C_{10}H_{10}O_4Cl_2$	45.31	45.59	3.80	3.84	.030	.021	> .210
17	$HOCH_2CH_2$[h]	61	168	G	$C_9H_{10}O_5$	54.66	54.24	5.10	5.10	.150	.150	> .210

[a] All esters were prepared by reaction of protocatechuic acid with the appropriate alcohol in the presence of sulfuric acid unless otherwise noted. [b] All melting points are uncorrected. [c] For recrystallization: A = petroleum ether (b. p. 65–110°); B = carbon tetrachloride; C = heptane; D = benzene–petroleum ether (b. p. 30–60°); E = benzene; F = petroleum ether (b. p. 30–60°); G = water. [d] Inhibiting concentrations were determined in accordance with the method described earlier.[5a] [e] Prepared by reaction of protocatechuic acid with appropriate alcohol in the presence of hydrogen chloride. [f] This is a previously known compound having been prepared by the hydrogen chloride procedure. [g] Prepared by reaction of protocatechuic acid with phenol in the presence of phosphorus oxychloride. Method patterned after preparation of phenyl salicylate by Siefert [*J. prakt. Chem.*, [2] **31**, 472 (1885)]. This compound was previously reported by Barger [*J. Chem. Soc.*, **93**, 569 (1908)] who hydrolyzed phenyl 3,4-carbodioxybenzoate with dilute ammonia. [h] Prepared by reaction of potassium protocatechuate with ethylene chlorohydrin.

TABLE II

ABSORPTION SPECTRA OF ESTERS OF PROTOCATECHUIC ACID

Number[a]	Maximum[b] λ	Maximum[b] *k*	Minimum[b] λ	Minimum[b] *k*	Maximum λ	Maximum *k*	Minimum λ	Minimum *k*	Maximum λ	Maximum *k*	Minimum λ	Minimum *k*	Maximum λ	Maximum *k*	Minimum λ	Minimum *k*	Breaks in curve, λ
1	2925	30.5	2775	17.5	2625	56.5	2620	56.0			2550	53.5	2530	54.2	[c]		2590, 2475[d]
2	2925	28.2	2775	16.8					2580	57.9					[c]		2550[e]
3	2925	26.7	2775	15.7					2570	54.9					[c]		2530
4	2950	31.8	2800	22.1	2625	55.0									2375	15.7	[f]
5	2925	25.0	2775	15.0					2580	51.5					[c]		2550, 2530
6	2925	24.9	2760	13.8					2580	51.0					[c]		2550
7	2925	23.8	2765	13.6	2625	41.9	2620	40.7	2590	46.6	2550	38.2	2530	40.7	2340	12.2	
8	2925	24.1	2760	13.9	2630	42.3	2620	41.3	2585	46.3	2550	38.3	2525	40.8	2325	12.7	[g]
9	2925	22.6	2762	13.0							2550	36.3	2525	38.0	2325	11.4	2630[h]
10	2950	27.4	2825	19.9	2650	53.5									2375	15.7	2570
11	2930	25.4	2775	15.2	2625	44.9	2620	43.0	2590	47.9	2550	38.2	2530	42.5	2340	12.7	[i]
12	2950	20.9	2800	16.6	2630	44.3	2610	44.1	2590	44.8	2550	32.8	2525	33.8	2350	10.0	
13	2925	26.9	2775	16.1	2640	47.2	2610	43.2	2590	47.8					2350	11.6	2570
14	2925	23.7	2775	14.2	2630	44.8	2610	45.8	2600	47.0					2350	10.2	2570
15	2950	22.3	2800	13.9	2640	40.8	2610	37.7	2590	40.3	2540	30.8	2530	32.1	2350	9.0	
16	2950	23.1	2800	14.5	2630	42.6									2350	8.4	2610, 2590[j]
17	2925	24.8	2775	15.0	2630	46.2	2620	43.0	2590	47.8	2550	37.6	2540	41.0	2350	12.8	2530

[a] These numbers correspond to the esters in Table I. [b] The wave length (λ) is an Ångström units and *k* = specific extinction. [c] This minimum was not recorded because spectral data were not obtained at sufficiently low wave lengths. There is no doubt that the minimum exists, however. [d] Additional maximum at 2575 (*k* = 60.1). [e] Additional minimum at 2570 (*k* = 57.2) and maximum at 2560 (*k* = 58.8). [f] Additional maximum at 2200 (*k* = 95.5) and minimum at 2125 (*k* = 82.3). This ester and No. 16 were the only esters whose absorption spectra were determined at such low wave lengths. [g] Additional maximum at 2640 (*k* = 42.1) and minimum at 2630 (*k* = 41.7). [h] Additional maximum at 2575 (*k* = 42.9). [i] Additional maximum at 2640 (*k* = 44.9) and minimum at 2630 (*k* = 42.5). [j] Additional maxima at 2240 (*k* = 59.5) and 2225 (*k* = 60.1) and minimum at 2230 (*k* = 59.1).

cations. If unpurified syringic acid obtained from crude trimethylgallic acid was used for esterification, the yield of ester was uniformly lowered.

In addition to esters of syringic acid, this paper reports analogous esters of protocatechuic acid which acid is easily prepared in high yield by the caustic fusion of vanillin at temperatures above 240°.[8]

The esters prepared and tested in this study comprise alkyl, aryl, and hydroxy-, alkoxy-, aryloxy- and halogeno-alkyl esters of syringic and pro-

(8) Pearl, THIS JOURNAL, **68**, 2180 (1946).

TABLE III

ESTERS OF SYRINGIC ACID

No.[a]	Yield, %	M. p. or b. p.[b] °C.	Mm.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Inhibiting concn.[c] *Bacillus mycoides*	Ultraviolet absorption Maximum[d] λ	Maximum[d] *k*	Minimum[d] λ	Minimum[d] *k*	Maximum λ	Maximum *k*
1[e,f]	90	107							>0.21						
2[g]	95	85-86[h]		$C_{11}H_{14}O_5$	58.40	57.84	6.24	6.09	> .21	2750	51.2	2400	6.5	2275	54.1
3	88	84-85		$C_{12}H_{16}O_5$	59.99	59.69	6.71	6.70	> .21						
4	91	66-67		$C_{12}H_{16}O_5$	59.99	60.05	6.71	6.75	.15						
5	90	67-68[i]		$C_{13}H_{18}O_5$	61.40	61.33	7.13	7.09	.21[j]						
6	91	66-67		$C_{13}H_{18}O_5$	61.40	61.40	7.13	7.14	.09						
7[k]	98	140-145	0.4[l]	$C_{14}H_{20}O_5$	62.67	62.77	7.51	7.62	.009	2750	47.8	2400	10.1	2170	103.4
9	60[m]	165-167	1.0[n]	$C_{15}H_{22}O_5$	63.81	63.69	7.86	7.86	.009						
10[o]	50	113		$C_{15}H_{14}O_5$	65.68	65.66	5.14	5.23	> .21	2875	51.5	2430	8.9	2125	96.1
13[f]	75	89		$C_{11}H_{13}O_5Cl$	50.68	50.67	5.03	5.09	> .21	2830[p]	46.3	2400	4.8	2200	88.3
15[f]	69	200-203	1.0[q]	$C_{12}H_{14}O_5Cl_2$	46.62	46.95	4.56	4.72	.21	2800	34.9	2410	4.6	2200	73.4
16[f]	76[r]	196-202	1.0[s]	$C_{12}H_{14}O_5Cl_2$	46.62	46.77	4.56	4.69	.09	2800	36.5	2410	4.5	2175	76.7
17[t]	26[m]	197	3.0[u]	$C_{11}H_{14}O_6$	54.54	53.49	5.83	5.93	> .21	2700	33.8	2375	6.2	2150	82.0
18	77	50-51		$C_{13}H_{18}O_5$	61.40	61.27	7.13	6.98	.09						
19	79	158-160	1.5[v]	$C_{14}H_{20}O_5$	62.67	63.02	7.51	7.85	.09	2700	36.0	2375	7.1	2200	82.1
20	82	145	1.1[w]	$C_{15}H_{22}O_5$	63.81	63.95	7.86	7.86	.009	2700	34.4	2375	4.7	2150	84.8
21[f]	46[m]	185-187	2.0[x]	$C_{13}H_{18}O_6$	57.77	57.11	6.71	6.71	> .21	2700	34.8	2400	6.7	2175	92.1

[a] The numbers refer to the same esters enumerated for protocatechuic acid in Table I. New esters in this table are; 18, *s*-butyl; 19, diethylcarbinyl; 20, methylisobutylcarbinyl; 21, 2-ethoxyethyl. [b] All compounds were recrystallized from petroleum ether (b. p. 65–110°) except where indicated. All melting points and boiling points are uncorrected. [c] Inhibiting concentrations were determined in accordance with method described earlier.[5a] The inhibiting concentration against *Aerobacter aerogenes* was 0.15% for diethylcarbinyl and 0.21% for isoamyl, but was greater than 0.21% for all other esters. The inhibiting concentration against *Aspergillus niger* was 0.15% for propyl but was greater than 0.21% for all other esters. [d] The wave length (λ) is in ångström units and *k* = specific extinction. [e] This is a previously reported compound; see Graebe and Martz, *Ber.*, **36**, 217 (1903). [f] Prepared by the reaction of syringic acid with the alcohol in the presence of hydrogen chloride. [g] Prepared by the reaction of syringic acid with the alcohol in the presence of sulfuric acid. All esters were prepared by this general method unless otherwise indicated. [h] Bogert and Plaut [THIS JOURNAL, **37**, 2728 (1915)] reported ethyl syringate as melting at 55.8°. It is possible that this was a typographical error and should have been 85.5°. [i] Recrystallized from dilute methanol. [j] Dissolved in ethanol for toxicity determination. [k] Bogert and Plaut reported isoamyl syringate as melting at 101° when prepared by the same procedure. Their results could not be reproduced. [l] $n^{29}D$ 1.5270. [m] Crude syringic acid prepared from crude trimethylgallic acid was employed in this esterification. [n] $n^{26}D$ 1.5164. [o] Prepared by the reaction of syringic acid and phenol in the presence of phosphorus oxychloride. [p] Break in curve at 2890 Å. [q] $n^{22}D$ 1.5589. [r] 44% by the hydrogen chloride method from crude syringic acid. [s] $n^{25}D$ 1.5563. [t] Prepared by the reaction of potassium syringate with ethylene chlorohydrin. [u] $n^{25}D$ 1.5458. [v] n^{25} 1.5287. [w] $n^{25}D$ 1.5139. [x] $n^{23}D$ 1.5343.

tocatechuic acids. These were prepared by the procedures employed for the corresponding vanillic acid esters reported earlier.[5a,b]

The inhibiting concentrations of these esters were determined for the three representative aerobic microörganisms—namely, non-spore-forming (*Aerobacter aerogenes*) and spore-forming (*Bacillus mycoides*) bacteria and molds (*Aspergillus niger*). Unlike their analogs the vanillates, the protocatechuic acid esters in low concentration inhibited *Aerobacter aerogenes*. In addition, toxicities toward *Bacillus mycoides* were generally very good. The esters and their toxicities are described in Table I.

Except for a few instances, the syringates were ineffective inhibitors of these organisms at the concentrations studied. Only the propyl ester inhibited the fungus *Aspergillus niger*, and only the diethylcarbinyl and isoamyl esters were effective against the non-spore-former, *Aerobacter aerogenes*. The propyl, isobutyl, *s*-butyl, butyl, diethylcarbinyl, 2,3-dichloropropyl, 2-chloro-1-chloromethylethyl, methylisobutylcarbinyl, and 2-ethylbutyl esters effectively inhibited the spore-former, *Bacillus mycoides*, but only the last two to any high degree.

The ultraviolet absorption spectra of the protocatechuic acid esters were determined in purified dioxane as described previously for vanillic acid derivatives.[1] A comparison with the absorption spectra of vanillic acid esters indicates that demethylation of vanillic acid has little effect on its ultraviolet absorbing characteristics. The ultraviolet absorption curve of methyl protocatechuate is reproduced in Fig. 1. The maxima and

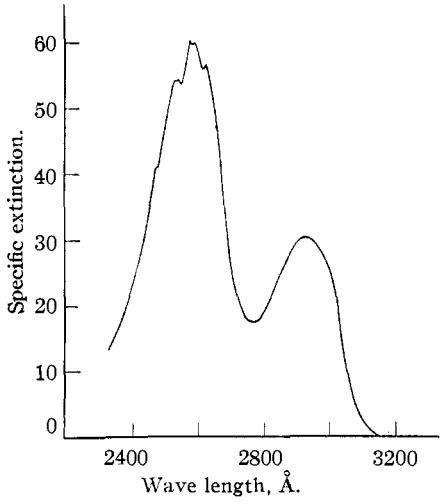

Fig. 1.—Ultraviolet absorption spectrum for methyl protocatechuate.

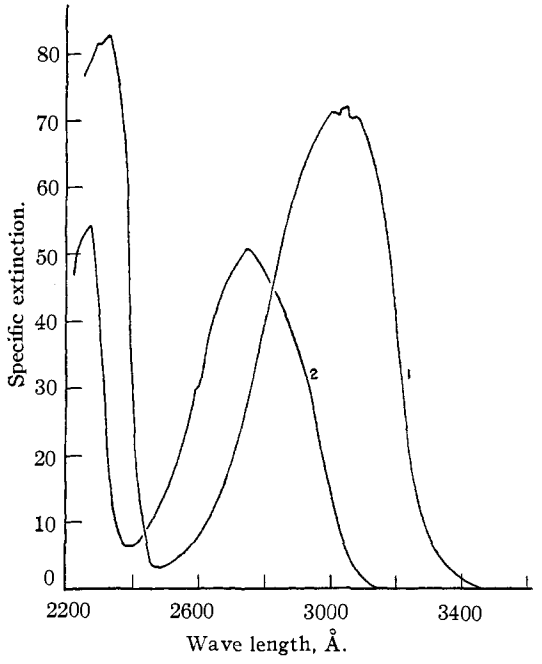

Fig. 2.—Ultraviolet absorption spectra for syringaldehyde (1) and syringic acid (2).

minima of the absorption spectra of the other esters are shown in Table II. Very little change in the fundamental protocatechuic acid absorption curve is effected by esterification with various alcohols or phenols.

The ultraviolet absorption spectra of the syringates were determined in 95% ethanol solution. The absorption spectrum for syringic acid is reproduced and compared with that of syringaldehyde in Fig. 2. As was the case in going from vanillic acid,[1] when syringaldehyde is oxidized to the acid, the absorption curve exhibits a hypsochromic shift. The principal absorption maxima for syringic acid and its derivatives are 2750 and 2280 Å. These compare with 3050 and 2325 Å., respectively, for syringaldehyde.[9] The maxima and minima of some of the esters are shown in Table III. Very little change in the fundamental syringic acid absorption curve is effected by esterification with various alcohols and phenols.

Acknowledgment.—The authors are indebted to the Microbiological and Analytical Departments of The Institute of Paper Chemistry for the toxicities and analyses reported in this paper.

Summary

A number of esters of syringic and protocatechuic acids have been prepared and tested for their toxicities toward representative microörganisms and for their ultraviolet absorption spectra.

(9) Pearl, THIS JOURNAL, **70**, 1747 (1948).

APPLETON, WISCONSIN RECEIVED MAY 21, 1949

[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

Studies on the Schardinger Dextrins. IV. The Action of Soy Bean Beta Amylase on Amyloheptaose[1]

BY DEXTER FRENCH, MELVIN L. LEVINE,[2] J. H. PAZUR[3] AND ETHELDA NORBERG[4]

In paper II of this series dealing with the preparation and properties of amyloheptaose[5] it was stated that this substrate was hydrolyzed by β-amylase to give two moles of maltose and one mole of a trisaccharide, thus indicating the heptasaccharide character of the initial hydrolytic product of Schardinger β-dextrin. Amyloheptaose is indeed an inviting test material for the examination of enzyme action, since it is a chemically defined oligosaccharide of the starch type; yet it is easily soluble in water, and it is free from the structural irregularities and inhomogeneities of natural starch. The present paper deals with the course and products of the action of soy bean β-amylase on amyloheptaose.

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(5) French, Levine and Pazur, THIS JOURNAL, **71**, 356 (1949).

In order to follow the hydrolytic action of β-amylase on amyloheptaose, the authors used at first conventional analytical methods including optical rotations and reducing determinations. Later it was found advantageous to use methods giving both qualitative and quantitative information; in particular the techniques of quantitative elution chromatography (Fig. 1) and electrophoretic analysis of the oxidized oligosaccharides (as the potassium aldonates, Fig. 2) have been of especial help in arriving at direct answers on the qualitative and quantitative composition of enzyme digestion mixtures.

Each of the methods mentioned above indicates that soy bean β-amylase hydrolyzes amyloheptaose to give two molecules of maltose and one molecule of amylotriose ("maltotriose"). The amylotriose has been isolated and characterized by its optical rotation, reducing value, and digestibility by salivary amylase (thus indicating "amylo" character). Amylotriose appears to be completely resistant to the action of β-amylase; under no cir-