anism[5,6] is different from the above-mentioned reactions. The dissociation reaction of cyclic ureas proceeds through the double transfer of protons with opening of the ring. In this mechanism, two nitrogen atoms in the ring change their coördination numbers; that is, the coördination numbers of nitrogen atoms change from three to two and three to four, respectively. These points are different from those of the SN1, SN2, free radical and carbonyl-type reactions. Accordingly, different reactivity is expected.

In chloroacetic acid of all ring ureas studied, the 8-membered ring urea dissociates most rapidly (k_1 210 $\times$ 10^{-3} min.$^{-1}$ at 139.5°) and the 5- dissociates most slowly (k_1 1.21 $\times$ 10^{-3}). Eleven- and large ring compounds dissociate moderately. Comparing the results with those of other ring compounds reported, they generally resemble the reactivity of SN1, SN2 and radical-type reactions.[14] Accordingly, the reactivity of ring ureas is in inverse relation with that of carbonyl-type reactions.[11] A characteristic result obtained in the present experiment is that 5- and 6- ring ureas have similar reactivity, which has not been observed in other reaction types.

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Electron Exchange Polymers. IX. Synthesis of Polymers of 2,5-Dihydroxyphenylalanine and of 3,4-Dihydroxyphenylalanine (DOPA)[1]

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Methods are given for the preparation by the N-carboxyl anhydride technique of high molecular weight polypeptides of 2,5-dihydroxyphenylalanine and 3,4-dihydroxyphenylalanine and of copolymers of each with L-glutamic acid. The methods include those for the preparation of the appropriate monomers in which the phenolic hydroxyls of the dihydroxyphenylalanines are protected by acetyl groups. These syntheses now make available new electron exchange polymers that are also polypeptides.

In the first paper of this series,[3] the possible usefulness of electron exchange polymers of suitable composition and structure as models for certain biological systems was suggested. It seemed desirable to investigate this possibility. The postulate on which this work was based is that a reactive group when incorporated in a polymeric structure will show one or more properties different in kind or different in quantity from those of the "same" group in the molecularly dispersed monomeric state. A number of investigations have been carried out effectively from this point of view. For example, the investigations of Haskell and Hammett[4] and the further investigations of Hammett and co-workers[5] fall in this category. These investigators compared the catalytic behavior of hydrochloric acid and the acid forms of cation exchangers toward the hydrolysis of esters. They found differences in rate and specificity effects. As another example from a growing literature, Morawetz and Zimmering[6] found that solvolysis of the ester group in acrylic acid–*p*-nitrophenyl methacrylate copolymers seemed to proceed by a mechanism different from that of the solvolysis of the ester group in the monomeric model *p*-nitrophenyl trimethylacetate. As a third example, Lautsch and his co-workers[7] have carried out extensive investigations of the changes in spectra, in catalytic properties and in oxidation–reduction potential that occur when a porphyrin group is incorporated into a large molecule. We have set out to examine the properties of hydroquinone and catechol groups when present as side-chains to a polypeptide structure, thus supplementing our earlier work.[8]

This paper reports the first steps in this investigation: the synthesis of polymers of 2,5-dihydroxyphenylalanine and 3,4-dihydroxyphenylalanine and copolymers of these two amino acids with L-glutamic acid by the N-carboxyl anhydride technique. Our synthesis is patterned after the polytyrosine synthesis reported by Schlögl, Wessely and Wawersich[9] and the polyserine synthesis reported by Frankel, Cordova and Breuer.[10]

2,5-Dihydroxyphenylalanine, (I), was treated

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(3) H. G. Cassidy, THIS JOURNAL, **71**, 402 (1949).

(4) V. C. Haskell and L. P. Hammett, *ibid.*, **71**, 1284 (1949).

(5) S. A. Bernhard and L. P. Hammett, *ibid.*, **75**, 1798, 5834 (1953); S. A. Bernhard, E. Garfield and L. P. Hammett, *ibid.*, **76**, 991 (1954); P. Riesz and L. P. Hammett, *ibid.*, **76**, 992 (1954).

(6) H. Morawetz and P. E. Zimmering, *J. Phys. Chem.*, **58**, 753 (1954).

(7) For bibliography see W. Lautsch, W. Broser, W. Biedermann and H. Gnichtel, *J. Polymer Sci.*, **17**, 479 (1955). W. Lautsch, W. Broser, E. Höfling, H. Gnirhtel, E. Schröder, R. Kruger, J. Woldt, G. Schulz, R. Wiechert, W. Bandel, G. Kurth, H.-J. Kraege, W. Gehrmann, K. Prater, G. Parsiegla, R. Pasedag and W. Hunger, *Kolloid Z.*, **144**, 82 (1955).

(8) For references see L. Lullinger and H. G. Cassidy, *J. Polymer Sci.*, **20**, 417 (1956); **22**, 271 (1956); and M. Ezrin and H. G. Cassidy, THIS JOURNAL, **78**, 2525 (1956).

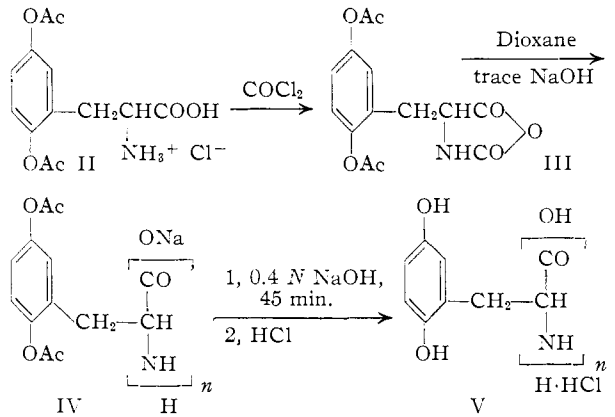
(9) K. Schlögl, F. Wessely and E. Wawersich, *Monatsh.*, **84**, 705 (1953).

(10) M. Frankel, S. Cordova and M. Breuer, *J. Chem. Soc.*, 1991 (1953); M. Frankel, M. Breuer and S. Cordova, *Experientia*, **8**, 299 (1952).

with acetyl chloride in the presence of glacial acetic acid saturated with hydrogen chloride, according to the procedure described by Bretschneider and Biemann[11] for the synthesis of O-acetyltyrosine hydrochloride, to form 2,5-diacetoxyphenylalanine hydrochloride (II). II was suspended in dioxane and treated with phosgene to form the desired monomer, 4-(2,5-diacetoxybenzyl)-2,5-oxazolidine dione (III). Polymerizations to IV were carried out in dioxane solution by initiation with 0.005 mole of sodium hydroxide in methanol per mole of monomer. Acetyl groups were cleaved by alkaline treatment in an inert atmosphere to give V. This method appears to be superior to that employing Overell and Petrow's hydrobromic–acetic acid reagent[12]; see Experimental.

Poly-DOPA was prepared through a synthetic sequence similar to I to V, except that it was more convenient to isolate the 3,4-diacetoxyphenylalanine as the hydrobromide than as the hydrochloride. The latter was characterized, but the former was used in the synthesis.

Copolymers were prepared by copolymerizing the above-described monomers with the N-carboxyl anhydride of γ-benzyl glutamate. Protecting groups were removed stepwise from the copolymers by first cleaving the benzyl esters with a saturated solution of hydrobromic acid in acetic acid, then completing the removal of acetyl groups in a separate step by alkaline treatment.



Katchalski and Sela[13] reported a polytyrosine synthesis which has advantages in the step where protective groups are removed. We found that the tricarbobenzoxy derivatives, intermediates in this synthesis, were inconvenient to work with. The tricarbobenzoxy derivative of 2,5-dihydroxyphenylalanine (N-benzyloxycarbonyl-β-(2,5-dibenzyloxycarbonyloxyphenyl)-D,L-alanine) was prepared and purified. A tricarbobenzoxy derivative of 3,4-dihydroxyphenylalanine could not be prepared, though a tricarbomethoxy derivative, m.p. 127°, was prepared in a small-scale run and characterized on the basis of its infrared spectrum. Attempts to repeat this synthesis failed to yield crystalline material. Apparently similar difficulty of this kind was encountered by O'Neill, Veitch and Wagner-Jauregg[14] in their attempts to prepare simple peptides of DOPA.

The molecular weights of the unhydrolyzed polymers were checked by end-group titrations.[15] Number average molecular weights between 5,000 and 10,000 were observed by this method. Intrinsic viscosity determinations of unhydrolyzed copolymers, determined in dichloroacetic acid (DCA), when compared with the data of Doty, Bradbury and Holtzer,[16] indicated weight average molecular weights in the range of 34,000 to 45,000. The unexpectedly large difference between the number and weight average molecular weights suggests that either the polymer preparations are of wide molecular weight distributions or that partial saponification of the phenolic ester groups during the polymerization led to a polymer having a branched structure. Support for the latter possibility is provided by the color of the polymerization mixtures (see Experimental), the fact that more amino[17] than carboxyl end groups were found in the polymer, the difficulty of titrating the dihydroxyphenylalanine monomers with alkaline reagents[15] and the known ready hydrolysis of acetylated phenolic groups in similar types of compounds.[18]

The intrinsic viscosity values observed for the simple polymers appear to be in better agreement with the number average molecular weights estimated from end-group titration results, although an exact assignment of weight average molecular weight values to these polymers cannot be made at present.

The difference in polydispersity between the simple polymers and the copolymers might suggest that the latter are polyblends; mixtures of relatively low molecular weight (5–6,000) polydiacetoxyphenylalanine and high molecular weight poly-γ-L-benzyl glutamate (*ca.* 50,000). Against this supposition is the observation that the hydrolyzed copolymers are completely soluble at *p*H 7.3, while poly-3,4-dihydroxyphenylalanine is insoluble at this *p*H. The differences in phenolic ionization behavior observed for the simple polymers and the copolymers is of interest in this connection, and we hope to report on this subsequently.

Methods for preparing these substances are presented because of the increasing interest in the catalytic and reagent properties of quinones. They provide reagents which are readily removable from a reaction mixture by precipitation or dialysis and which, like other oxidation–reduction polymers based on quinones,[3] contribute to or remove from the reaction only electrons and protons. Investi-

(11) H. Bretschneider and K. Biemann, *Monatsh.*, **81**, 647 (1950).

(12) B. G. Overell and V. Petrow, *J. Chem. Soc.*, 232 (1955).

(13) E. Katchalski and M. Sela, THIS JOURNAL, **75**, 5284 (1953).

(14) J. J. O'Neill, F. P. Veitch and T. Wagner-Jauregg, Abstract of paper 151, presented before the Division of Organic Chemistry, 126th Meeting, A.C.S., New York, September, 1954.

(15) M. Sela and A. Berger, THIS JOURNAL, **77**, 1893 (1955); A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1554 (1953).

(16) P. Doty, J. H. Bradbury and A. M. Holtzer, THIS JOURNAL, **78**, 947 (1956). The comparison is believed to be justified since copolymer contained about 90% of γ-benzyl glutamate.

(17) These values have been corrected for the amount of NaOH initiator added to the polymerization mixtures. The –COONa end-group on the polymers formed as a result of the initiation step would be counted as amine end-groups if this correction were not made. We thank Mr. R. D. Lundberg for calling this to our attention.

(18) C. R. Harington and S. S. Randall, *Biochem. J.*, **25**, 1028 (1931).

gation of the reagent and catalytic properties of these substances is being continued.

Experimental

N-Benzyloxycarbonyl-β-(2,5-dibenzyloxycarbonyloxyphenyl)-D,L-alanine.—An ice-cold solution of 4.01 g. of 2,5-dihydroxyphenylalanine[19] in 38 ml. of 0.74 *N* sodium hydroxide was treated slowly under nitrogen, with rapid stirring, with 12.2 g. of 93.5% benzyl chloroformate and 15.0 ml. of cold 4 *N* sodium hydroxide added simultaneously. The mixture became turbid and a heavy oily substance deposited upon the sides of the flask. Ether, 10 ml., was then added to help disperse the mixture. Stirring was continued for 2.5 hr., and the reaction mixture was diluted with ether. Three fractions were obtained: aqueous and ether phases, and the insoluble sodium salt of the tricarbobenzoxy derivative. The aqueous and sodium salt layers were acidified with 4 *N* HCl to congo red. The ether layer was extracted further with 5% sodium bicarbonate solution. The aqueous and sodium salt layers were again separated from the ether and acidified with 4 *N* hydrochloric acid. The aqueous and sodium salt layers could be made miscible if the mixture was diluted with distilled water. After several days in the refrigerator, the oily product solidified as a light brown mass. The material was filtered and washed with distilled water. The yield was 9.6 g. (78%).

The material is very difficult to purify. Repeated recrystallization from a dilute solution in 30% acetic acid led to 2.0 g. of small, white needles, m.p. 126.7–127.8°.

Anal. Calcd. for $C_{33}H_{28}O_{10}N$ (600): C, 66.09; H, 4.86; N, 2.34; neut. equiv., 600. Found: C, 65.95; H, 4.98; N, 2.30; neut. equiv., 600.6, 596.1.

Crystalline material may be obtained if the gummy mass which is often obtained from either the reaction mixture or the purification process is partially dissolved in ether. Some crystalline material is usually left undissolved. The ether solution on standing usually begins to deposit crystalline material after several days. The crystals thus obtained are cubic, and the substance melts at 169–170°. The cubic crystals are not appreciably soluble in chloroform whereas those in the needle form, melting at 127–8°, are readily soluble. Both types of crystals show almost identical infrared spectra in Nujol mulls. The soluble form, in chloroform solution, showed ester absorption at 5.68 μ and carboxyl absorption at 5.82 μ.

Regeneration of 2,5-Dihydroxyphenylalanine.—Purified tricarbobenzoxy derivative, 72.9 mg., was dissolved in 2 ml. of glacial acetic acid, and 3.7 ml. of a 50% solution (by weight) of hydrogen bromide in glacial acetic acid was added. After an hour, the solution was swamped with ether and the crystalline 2,5-dihydroxyphenylalanine hydrobromide was isolated. The yield was 30.7 mg. (91%), m.p. 272–273° dec. The infrared spectrum in Nujol was identical with that of an authentic sample of 2,5-dihydroxyphenylalanine hydrobromide prepared directly from the pure amino acid and the hydrobromic–acetic acid reagent, m.p. 274.5–275° dec.

β-(2,5-Diacetoxyphenyl)-D,L-alanine Hydrochloride.—Five grams of β-(2,5-dihydroxyphenyl)-D,L-alanine was added to 30 ml. of glacial acetic acid, previously saturated with dry hydrogen chloride. The amino acid dissolved and after a short time its salt settled out. Acetyl chloride, 25 ml., was added either to the solution or the suspension, and the mixture was allowed to stand overnight at room temperature, whereupon 30 ml. of the hydrochloric–acetic acid reagent and 30 ml. of acetyl chloride were added and the reaction mixture heated quickly to 75° with stirring. The mixture was allowed to cool and was then swamped with anhydrous ether. The crude β-(2,5-diacetoxyphenyl)-D,L-alanine hydrochloride after several hours quickly solidified, and was collected, washed with ether and allowed to dry in air. The yield was 5.3 g. (66%), m.p. 158–159°.

For analysis the sample was recrystallized from a methanol–ether mixture, giving colorless needles, m.p. 154–155° (after drying in the Abderhalden apparatus, m.p. 160–161°).

Anal. Calcd. for $C_{13}H_{16}O_6NCl$ (317.7): C, 49.12; H, 5.09; N, 4.41; Cl, 11.16. Found: C, 48.68; H, 5.74; N, 4.75; Cl, 10.81.

(19) H. B. Gillespie, *Biochem. Prep.*, **3**, 79 (1953).

The compound in a Nujol mull showed ester absorption in the infrared region at 5.65 μ and carboxyl absorption at 5.72 μ.

The compound is soluble in water, pyridine, methanol and is insoluble in chloroform, benzene and ether. It gives negative tests with ferric chloride, Millon reagent and iodine in potassium iodide.

4-(2,5-Diacetoxybenzyl)-2,5-oxazolidine Dione.—Phosgene was slowly bubbled into a rapidly stirred suspension of 4.8 g. of β-(2,5-diacetoxyphenyl)-D,L-alanine hydrochloride in 50 ml. of dry purified dioxane, maintained between 40 and 60°. After about 2 or 3 hr. all of the salt had dissolved and the flask was flushed with dry nitrogen for 0.5 hr. to remove unreacted phosgene. The dioxane was then removed by distillation *in vacuo* leaving a colorless sirup, which was dissolved in 50 ml. of ethyl acetate, and the ethyl acetate was removed by distillation *in vacuo*. This process was repeated and the sirupy residue was taken up in a small amount of ethyl acetate. The crude anhydride was precipitated from solution by the addition of a large excess of ligroin. The oily residue which deposited usually solidified upon standing for several hours; yield was 3.4 g. (73.5%).

Several recrystallizations from ethyl acetate–petroleum ether mixtures yielded 2.8 g. of nearly colorless needles, m.p. 132.3–134.0°. The material showed a negative ferric chloride test. It is soluble in chloroform, ethyl acetate, dioxane and methanol and is insoluble in petroleum ether. The compound showed ester absorption in the infrared region: at 5.65 μ and anhydride absorption at 5.35 and 5.57 μ.

Anal. Calcd. for $C_{14}H_{13}O_7N$ (307): C, 54.73; H, 4.26; N, 4.55; acetyl, 28.01. Found: C, 54.94; H, 4.49; N, 4.53; acetyl, 28.23, 28.62.

β-(3,4-Diacetoxyphenyl)-D,L-alanine Hydrochloride.—One gram of 3,4-dihydroxyphenylalanine (Eastman No. 4915) was dissolved in 6 ml. of glacial acetic acid saturated with hydrogen chloride; 5 ml of acetyl chloride was then added carefully with swirling. A gummy precipitate formed and redissolved upon the addition of each increment of acetyl chloride. After the vigorous reaction subsided, a precipitate appeared. The mixture was allowed to stand overnight, when 6 ml. of the hydrochloric–acetic acid reagent and 5 ml. of acetyl chloride were added and the mixture was warmed quickly to 70° with stirring. Upon cooling and swamping the mixture with ether, 1.06 g. (66%) of crude β-(3,4-diacetoxyphenyl)-D,L-alanine hydrochloride was obtained, m.p. 186–187°. The material was recrystallized from a methanol–ether mixture to give white needles, m.p. 193–194°.

Anal. Calcd. for $C_{13}H_{16}O_6NCl$ (317): C, 49.12; H, 5.09; N, 4.41; Cl, 11.16. Found: C, 49.48; H, 5.15; N, 4.36; Cl, 10.90.

The substance shows a negative ferric chloride test and a very strong bicarbonate test. It is soluble in water, methanol, ethanol and pyridine. It is insoluble in ether, benzene and only slightly soluble in acetic acid.

When a saturated solution of hydrogen bromide in glacial acetic acid is used in the above preparation in place of the hydrogen chloride–acetic acid reagent, a quantitative yield of the corresponding hydrobromide is obtained. The hydrobromide was used in further synthetic work but was not characterized since it was probably contaminated with the corresponding hydrochloride.

Both materials showed similar infrared spectra in Nujol mulls. Peaks occurring at 5.65 μ (phenolic ester) and 5.76 μ (carboxyl) were observed.

4-(3,4-Diacetoxybenzyl)-oxazolidine-2,5-dione.—Finely ground β-(3,4-diacetoxyphenyl)-D,L-alanine hydrobromide, 4.5 g., was mixed with 100 ml. of purified dioxane, and phosgene was bubbled slowly into the rapidly stirred suspension kept at 50–70° until all of the amino acid salt went into solution. This required 3 to 4 hr. The red solution was heated for 0.5 hr. longer and was then flushed with dry nitrogen for 1 hr. to displace unreacted phosgene. The solution was then concentrated to a viscous sirup under nitrogen *in vacuo* (40–60°). The sirup was taken up in ethyl acetate and precipitated by adding petroleum ether slowly. Scratching and allowing the mixture to stand for several hours usually led to the solidification of the crude anhydride. In some cases it was necessary to repeat the above process before solid material was obtained.

Several recrystallizations from ethyl acetate–petroleum ether mixtures at room temperature led to 2.90 g. (75%) of

pure 4-(3,4-diacetoxybenzyl)-oxazolidine-2,5-dione as granular colorless crystals, m.p. 116–117° (swelling in sealed tube).

Anal. Calcd. for $C_{14}H_{13}NO_7$ (307.3): C, 55.06; H, 4.45; N, 4.52; acetyl, 28.01. Found: C, 54.73; H, 4.26; N, 4.55; acetyl, 28.13.

When 50.1 mg. of the material was heated slowly to 135° at 0.1 mm., it lost 7.3 mg. of CO_2. The calculated value for the weight loss during thermal polymerization was 7.2 mg. The material is soluble in ethyl acetate, hot benzene, ether, chloroform and dioxane. It is insoluble in petroleum ether and water. The compound shows infrared absorption in the carbonyl region at 5.31 and 5.55 mμ. Apparently ester and anhydride bands overlap at 5.55 μ for this compound.

When β-(3,4-diacetoxyphenyl)-D,L-alanine hydrochloride is used as the starting material in this preparation, identical material is obtained in 72% yield.

Poly-β-(2,5-diacetoxyphenyl)-D,L-alanine.—A solution of 2.75 g. of 4-(2,5-diacetoxybenzyl)-oxazolidine-2,5-dione in 35 ml. of purified dioxane was mixed with a solution of 0.45 ml. of 0.14 *N* sodium hydroxide in absolute methanol dissolved in 35 ml. of purified dioxane.

The mixture, on standing for one week, became light yellow in color. It was concentrated to one third its volume under reduced pressure at 40–50°. The polymer was then precipitated from solution by the addition of petroleum ether. The yellow sticky polymer was then taken up in ethyl acetate and reprecipitated by the addition of petroleum ether. The polymer was now cream in color and was no longer sticky. It was dissolved in a mixture of 125 ml. of redistilled benzene and 40 ml. of dioxane. The solution was filtered and freeze dried, yielding 2.23 g. (91%) of poly-2,5-diacetoxyphenylalanine.

The polymer thus obtained was fluffy and very friable but became very hard and dense during storage. Material which was convenient to work with was obtained by dissolving the polymer in glacial acetic acid and freeze-drying this solution. The light yellow powder thus obtained was dried *in vacuo* over sodium hydroxide to remove the last traces of acetic acid.

Anal. Calcd. for $(C_{13}H_{13}O_5N)_n$: C, 59.31; H, 4.98; N, 5.32. Found: C, 58.97; H, 5.18; N, 5.07; amino end-group titrations showed the polymer to have a number average mol. wt. of 11,000; [η] in DCA at 25°, 0.08.

The polymer appears to be somewhat hygroscopic, particularly when the last traces of dioxane or acetic acid have not been removed.

Biuret and picric acid[20] tests on this material were inconclusive due to the ready hydrolysis of the acetoxy groups and the accompanying discoloration of the liberated hydroquinone groups under the strong alkaline conditions required for these tests. The ninhydrin test, in contrast to most polypeptides, appeared to be negative.

The polymer showed ester absorption in the infrared region at 5.68 μ, and amide absorption at 6.00 and 6.46 μ.

Poly-β-(3,4-diacetoxyphenyl)-D,L-alanine.—A solution of 3.80 g. of 4-(3,4-diacetoxybenzyl)-oxazolidine-2,5-dione in 75 ml. of purified dioxane was mixed with a solution of 0.35 ml. of 0.186 *N* sodium hydroxide in anhydrous methanol dissolved in 50 ml. of dioxane. The mixture became light yellow overnight and was allowed to stand four days.

The polymerization solution was then filtered and concentrated to one-third its volume under nitrogen *in vacuo* (40–60°). The crude caramel-colored sticky polymer was obtained by precipitating it from solution by the addition of petroleum ether. A small amount of starting material (10–20 mg.) was isolated from the supernatant which was decanted from this precipitated polymer. This indicates that a somewhat longer polymerization time would perhaps have been advisable.

The crude polymer dissolved in ethyl acetate was treated with Norite and filtered. The polymer was then reprecipitated by adding petroleum ether. The light, cream-colored powder that separated from solution was then partially dissolved in fresh ethyl acetate, and enough petroleum ether was added to make the resulting solution contain 15% petroleum ether. The polymer was now dissolved in purified glacial acetic acid and freeze dried. The yield of poly-β-(3,4-diacetoxyphenyl)-D,L-alanine was 2.30 g. (67.4%). The polymer was obtained as a faintly yellow powder which did not appear to be hygroscopic, in contrast to the behavior of its 2,5-isomer.

The material showed a negative picric acid test. Heating the polymer with a ninhydrin solution caused the polymer to become blue in color, leaving a colorless solution. The biuret test was inconclusive, the catechol groups liberated in the alkaline solution apparently reduced the cupric reagent. The polymer showed ester absorption in the infrared region at 3.65 μ and monosubstituted amide absorption at 5.99 and 6.48 μ.

Anal. Calcd. for $(C_{13}H_{13}O_5N)_n$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.22; H, 5.17; N, 5.44; end amino titration showed the polymer to have a mol. wt. of 7000; [η] in DCA, 0.10

Poly-[90% γ-benzyl L-glutamate; 10%-β-(2,5-diacetoxyphenyl)-D,L-alanine].—A solution of 3.29 g. of benzyl L-4-β-(2,5-diketoöxazolidinyl)-propionate[21] (γ-benzyl N-carboxyl-L-glutamate anhydride) and 0.429 g. of 4-(2,5-diacetoxybenzyl)-oxazolidine 2,5-dione in 50 ml. of purified dioxane was diluted with 75 ml. of dioxane to which 0.69 ml. of 0.1 *N* sodium hydroxide in methanol had been added.

The polymerization mixture was allowed to stand for two weeks. Most of the dioxane was then removed by distillation under reduced pressure (40–60°) and the sirupy residue was treated with petroleum ether to precipitate the sticky, faintly green polymer. The material was then dissolved in ethyl acetate and partially reprecipitated by the addition of petroleum ether. The powdery precipitate was then dissolved in redistilled benzene to form a very viscous solution. This solution was treated with Norite, filtered and freeze-dried yielding 2.50 g. of the white spongy copolymer.

Anal. Calcd. for $[(C_{12}H_{13}O_3N)_9(C_{13}H_{13}O_5N)]_n$: C, 64.7; H, 5.80; N, 6.23. Found: C, 63.95; H, 5.86; N, 5.08; end carboxyl and amino group determinations gave number average mol. wts. of 7,500 and 5–6,000, respectively; [η] in DCA at 25°, 0.28 corresponding to a weight average mol. wt. of 34,000; $[\alpha]^{25}D$ +15.0 (*c* 5.62 mg./ml. in dioxane).

The material gave a negative picric test, appeared to give a negative ninhydrin test and showed a strongly positive biuret test only after being precipitated from dichloroacetic acid solution with water.

Poly-[90% γ-benzyl L-glutamate; 10% β-(3,4-diacetoxyphenyl)-D,L-alanine].—A solution of 4.50 g. of benzyl L-4-β-(2,5-diketoöxazolidinyl)-propionate[21] and 0.595 g. of 4-(3,4-diacetoxybenzyl)-oxazolidine 2,5-dione in 75 ml. of purified dioxane was mixed with a solution of 0.95 ml. of 0.1 *N* sodium hydroxide in anhydrous methanol dissolved in 100 ml. of purified dioxane. The solution became light pink immediately and became faintly blue in a few minutes. After several hours it developed a greenish cast. After three weeks the polymerization mixture was concentrated to one-half its volume under reduced pressure (50–60°). The crude polymer was precipitated as a very pale yellow sticky solid by the addition of petroleum ether.

The mixture was allowed to stand overnight and the supernatant liquid was decanted. The polymer was then taken up in ethyl acetate, treated with Norite and filtered. The polymer was then partially reprecipitated by the addition of petroleum ether. It was obtained as a colorless powder which when filtered was difficult to separate cleanly from filter paper. The polymer was then taken up in redistilled benzene and the resulting very viscous solution was filtered and freeze-dried.

The material was obtained as a white spongy mass which became easily charged when rubbed with glass. The yield was 2.8 g. (66%).

Anal. Calcd. for $[(C_{12}H_{13}O_3N)_9(C_{13}H_{13}O_5N)]_n$: C, 64.7; H, 5.79; N, 6.23. Found: C, 63.85; H, 5.76; N, 5.35; end carboxyl and amino group titrations showed the polymer to have number average mol. wts. of 8,900 and 5,500, respectively; [η] in DCA at 25°, 0.33 corresponding to a weight average mol. wt. of 45,000; $[\alpha]^{25}D$ +13.2 (*c* 7.04 mg./ml. in dioxane); $[\alpha]^{20}D$ +18.1 (*c* 32.0 mg./ml. in ethyl acetate).

The material showed a negative picric acid test. After being precipitated from dichloroacetic acid with water, the polymer gives a strongly positive biuret test. When the

(20) E. Abderhalden and E. Komm, *Z. physiol. Chem.*, **139**, 181 (1924).

(21) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

polymer is boiled with ninhydrin, the polymer becomes blue in color but the solution remains colorless. The polymer showed ester absorption in the infrared region at 5.78 μ and monosubstituted amide absorption at 6.06 and 6.47 μ.

Poly-β-(2,5-dihydroxyphenyl)-D,L-alanine.—Poly-β-(2,5-diacetoxyphenyl)-D,L-alanine, 285 mg., was mixed with 25 ml. of 0.4 *N* sodium hydroxide in a nitrogen atmosphere. After 0.5 hr. the polymer had completely dissolved. The solution was allowed to stand 45 minutes longer and was then acidified with 1 ml. of concd. hydrochloric acid. The resulting light yellow solution was deionized by passage through a 20-g. Amberlite 1R-100 column. The polymer was isolated by freeze-drying. To ensure complete removal of inorganic material, the freeze-dried product was dissolved in distilled water and the resulting solution was passed through a fresh Amberlite 1R-100 column. The polymer was again isolated by freeze-drying. The polymer was dried 12 hr. at 80° (0.1 mm.) over sodium hydroxide for analysis.[22] The polymer is very hygroscopic and must be stored in a desiccator.

Anal. Calcd. for $(C_9H_9O_3N)_n$: C, 60.33; H, 5.06; N, 7.82. Found: C, 53.87; H, 4.01; N, 5.27; acetyl, 11.13.[23]

Poly-β-(3,4-dihydroxyphenyl)-D,L-alanine (Poly-DOPA).—Five hundred mg. of poly-β-(3,4-diacetoxyphenyl)-D,L-alanine was covered with 35 ml. of 0.4 *N* sodium hydroxide. During this time the flask was flushed out with a stream of nitrogen to prevent oxygen from reacting with the liberated hydroquinone groups. The polymer was difficult to wet but went into solution in a period of 0.5 hr. The light brown solution was allowed to stand 45 minutes longer and was then filtered and acidified with concentrated hydrochloric acid. The precipitated polymer was centrifuged and washed five times with distilled water. The polymer was almost white in color at this stage. A small amount of sticky material was taken up in absolute ethanol and reprecipitated by the addition of water. The material thus obtained was fine and powdery. The polymer was then dried over sodium hydroxide at 0.1 mm. and 80° for 12 hr. The yield was 142 mg. (48%).

Anal.[22] Calcd. for $(C_9H_9O_3N)_{20}$: C, 60.33; H, 5.06; N, 7.82; NH_2, 0.45. Found: C, 59.22; H, 5.30; N, 5.80; NH_2, 0.56; acetyl, 1.65.

The material is not appreciably soluble in water in contrast to its 2,5-isomer and does not appear to be hygroscopic. The material showed a negative picric acid test and an inconclusive biuret test similar to its parent acetylated derivative. One of the reviewers kindly pointed out that comparison of the ratio of found N (5.80) to found NH_2 (0.56) with that calculated for the assumed degree of polymerization suggests that some degradation had occurred during deacetylation.

Poly-[90% L-glutamic acid; 10% β-(2,5-dihydroxyphenyl)-D,L-alanine].—Poly-[90% L-γ-benzyl glutamate; 10%-β-(2,5-diacetoxyphenyl)-D,L-alanine], 336 mg., was mixed with 10 ml. of a freshly saturated (50% by weight) solution of anhydrous hydrogen bromide in purified glacial acetic acid. The polymer swelled and went almost completely into solution during the first hour the mixture was allowed to stand. One hour later solid material had begun to separate from solution again. The mixture was allowed to stand overnight in a calcium chloride-dried atmosphere. The next day the mixture was swamped with ether and centrifuged. The precipitate was washed with ether and the mixture was centrifuged. This process was repeated six or more times until the ether washings showed a negative test for acid.

The swollen gelatinous polymer was then dried to a white amorphous powder and dried in the Abderhalden apparatus at 80° and 0.1 mm. over sodium hydroxide to remove the last traces of acetic acid, ether and hydrogen bromide. It is important that hydrogen bromide be completely removed before the polymer is exposed to the atmosphere, otherwise colored material will be produced. The yield was 189 mg. (94.6%).

Anal.[22] Calcd. for $[(C_5H_7O_3N)_9(C_9H_9O_3N)]_n$: C, 48.3; H, 5.4; N, 10.4. Found: C, 48.00; H, 5.33; N, 9.43; acetyl, 2.50.

The material gave a positive biuret test. Hydrolysis could be completed by allowing the polymer to stand for 1 hr. in 0.2 *N* sodium hydroxide, whereupon its acetyl content fell to 0.92%.

Poly-[90%-L-glutamic acid; 10%-β-(3,4-dihydroxyphenyl)-D,L-alanine].—One gram of poly-[90% γ-benzyl-L-glutamate; 10% β-(3,4-diacetoxyphenyl)-D,L-alanine] was suspended in 5 ml. of purified glacial acetic acid, and the suspension was warmed to 45°; 25 ml. of a freshly prepared, saturated (50% by weight) solution of anhydrous hydrogen bromide in purified glacial acetic acid was then added and the mixture was allowed to stand in a calcium chloride dried atmosphere. After the first hour nearly all had gone into solution and approximately 1 hr. later white material began to separate from solution. After 18 hr. the mixture was swamped with ether and centrifuged. The supernatant liquid was decanted, the polymer was washed with fresh ether and the mixture was again centrifuged. This process was repeated five or six times until the ether washings showed a negative test for acid.

The swollen gelatinous polymer was then dried in the Abderhalden apparatus over sodium hydroxide at 80° and 0.1 mm. to ensure removal of the last traces of acetic acid, hydrogen bromide and ether. The yield of white powdery material was 540 mg. (90%). The material is slightly hygroscopic and was stored in a desiccator.

Anal. Calcd. for $[(C_5H_7O_3N)_9(C_9H_9O_3N)]_n$: C, 48.3; H, 5.4; N, 10.4. Found: C, 47.99; H, 5.26; N, 8.88; acetyl 1.09; $[\alpha]^{25}D$ −68.5° (*c* 5.522 mg./ml. in *p*H 7.3 phosphate buffer); $[\alpha]^{29}D$ −60.4° (*c* 4.70 mg./ml. in *p*H 7.3 phosphate buffer).

The material showed a negative picric acid test, an apparent negative ninhydrin test and a very strong biuret test. The infrared spectrum of this polymer in a Nujol mull was difficult to interpret because the bands were very broad and diffuse. Carboxylic acid (5.80 μ) and monosubstituted amide (6.05) and 6.53 μ) bands were observed, however.

A sample of the product of the hydrobromic–acetic hydrolysis, 176 mg., was mixed under nitrogen with 10 ml. of 0.35 *N* sodium hydroxide, and the solution was allowed to stand 1 hr. at room temperature. The solution was then acidified with 4 *N* hydrochloric acid, and the precipitate that formed was centrifuged and washed with distilled water until the washings were neutral. The gelatinous precipitate was dried at 80° and 0.1 mm. over sodium hydroxide pellets for 16 hr.; yield 138 mg. The polymer treated in this way analyzed for 4.46% "acetyl."[22,23] Resuspended in distilled water and washed six to eight times with fresh portions of distilled water, and dried as above, the polymer analyzed for 0.0% acetyl.

It was difficult to remove acetyl completely from the acetylated polymers. The hydrobromic acid–acetic acid

TABLE I

SUMMARY OF ACETYL ANALYSES[23]

Hydrolysis conditions	Acetyl, % 2,5-Series	Acetyl, % 3,4-Series
Pure polymers		
a, 50% HBr–HOAc overnight at RT[a]	...	1.55
b, 0.2 *N* NaOH, 1 hr. under N_2 at RT	...	13.9
c, 0.4 *N* NaOH, 1 hr. under N_2 at RT	11.3	1.65
d, 0.2 *N* NaOH, 1 hr. under N_2 at RT followed by 0.4 *N* NaOH, 1 hr. under N_2	0.0	...
Copolymers (γ-benzyl glutamate)		
a, 50% HBr–HOAc overnight at RT	2.50	1.09
b, 50% HBr–HOAc overnight at RT followed by 0.2 *N* NaOH, 1 hr. under N_2 at RT	0.92	...
c, 50% HBr–HOAc overnight at RT followed by 0.4 *N* NaOH, 1 hr. under N_2 at RT	...	4.46
d, Above product rewashed with distilled water	...	0.0

[a] RT is room temperature.

(22) The hydrolyzed polymers, in general, were difficult to purify and gave poor analytical results, it being difficult to remove inorganic material and to dry them.

(23) Experimental "acetyl" values tend to be high due to some hydrochloric acid (bound by end amino groups) in the distillate

reagent of Overell and Petrow[12] was fairly effective. There was some indication that more vigorous conditions were required for the 2,5-series than the 3,4-series. The polymers prepared by this method of deacetylation analyzed poorly for carbon, hydrogen and nitrogen. A summary of results is given in Table I.

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NEW HAVEN, CONN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF VIRGINIA]

2-Substituted Derivatives of 3,4-Dihydroxyphenylalanine

BY CARL KAISER[1] AND ALFRED BURGER

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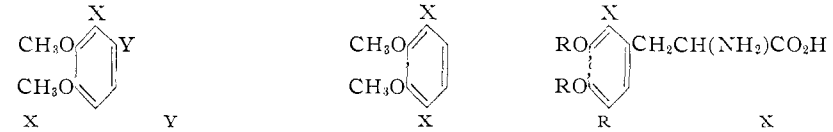
Because 2-methyl- and 2-chloro-3,4-dihydroxyphenylalanine inhibited the multiplication of influenza virus in the allantoic fluid of the chick embryo, the following additional 2-substituted derivatives of DOPA and their dimethyl ethers have been synthesized for biological screening: 2-bromo-, 2-fluoro-, 2-ethyl- and 2-nitro-DOPA. In addition, the synthesis of β-(2-methyl-3,4-dihydroxyphenyl)-N-methyl-α-alanine and β-(2-chloro-3,4-dihydroxyphenyl)-β-alanine has been performed.

3,4-Dihydroxyphenylalanine (DOPA) is the substrate of several enzyme systems such as dopa oxidase and dopa decarboxylase which convert it to melanins[2] and to 3,4-dihydroxyphenethylamine and norepinephrine.[3,4] These reactions play an important role in animal and plant metabolism and have been studied extensively *in vitro* and *in vivo*. Interference with these reactions should prevent the accumulation of the respective reaction products in certain pathogenic conditions and for this purpose inhibitors for dopa decarboxylase have been sought repeatedly.[5-10] When 2-methyl-3,4-dihydroxyphenylalanine[11,12] became available, it was included in a series of some two hundred compounds in tests for dopa decarboxylase inhibition *in vitro*, and the same substance and its 2-chloro analog[11] were screened for anti-phenylalanine behavior in various biological systems. Since several analogs of amino acids had been found to possess antiviral activity,[13,14] it seemed desirable to test the effect of both compounds against influenza virus. In the course of these experiments it was observed that both compounds inhibited the multiplication of the PR-8 strain of influenza virus in the allantoic fluid of the chick embryo.[15] Although in rats infected with the virus these two compounds were inactive, additional 2-substituted derivatives of 3,4-dihydroxyphenylalanine were prepared with the hope of overcoming this obstacle.



X	Y	X	R	X
		Br (I)	CH_3	Br (IV)
Br	CH_2Cl (II)	F (VI)	CH_3	F (IX)
F	CH_2Cl (VII)	C_2H_5 (XIX)	CH_3	C_2H_5* (XXII)
C_2H_5	CH_2Cl* (XX)		CH_3	NO_2 (XIII)
NO_2	CH_2Cl (XI)		CH_3	CH_3 (XXVI)
Br	$CH_2C(NHCOCH_3)(CO_2C_2H_5)_2$ (III)		H	Br (V)
F	$CH_2C(NHCOCH_3)(CO_2C_2H_5)_2$ (VIII)		H	F (X)
C_2H_5	$CH_2C(NHCOCH_3)(CO_2C_2H_5)_2$* (XXI)		H	C_2H_5* (XXIII)
NO_2	$CH_2C(NHCOCH_3)(CO_2C_2H_5)_2$ (XII)		H	NO_2 (XIV)

* The position of this group has not been confirmed conclusively.

Fig. 1.

For the synthesis of 2-bromo-3,4-dihydroxyphenylalanine, 3-bromoveratrole (I)[16] was chloromethylated to 2-bromoveratryl chloride (II); this was condensed with diethyl acetamidomalonate to give III which was hydrolyzed to β-(2-bromo-3,4-dimethoxyphenyl)-α-alanine (IV) with hydrochloric acid. β-(2-Bromo-3,4-dihydroxyphenyl)-α-alanine (V) was obtained by ether cleavage with 47% hydriodic acid. By an analogous sequence (VI → VII → VIII → X) 3-fluoroveratrole (VI)[17] was transformed into β-(2-fluoro-3,4-dihydroxy-

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