water-soluble, polycarboxylic acids.[1] Recent studies in this Laboratory of the reaction products from decarboxylation of the copper salts of these acids have resulted in the recovery of the lactone of 2′-hydroxy-2-biphenylcarboxylic acid (6-dibenzopyrone), along with mono- and bicyclic aromatic hydrocarbons. This lactone was characterized by melting point, ultimate analysis, and comparison of infrared spectrum, of ultraviolet spectrum, and of properties of the methoxy acid derivative with those of an authentic sample.

The isolation of this lactone from the oxidation products of coal is highly suggestive in connection with oxidation mechanisms of carbonaceous materials. Lactone rings are very sensitive to *p*H. In an alkaline hydroxide solution, the ring is opened and the resultant hydroxyl and carboxyl groups will undergo the usual reactions of such groups. In an acid medium, a stable six-membered oxygen-containing ring is formed and further attack on interior rings would be expected to be difficult. The presence of lactones as intermediates would furnish a possible explanation for the much higher rates of oxidation of coals in alkaline than in acid media. Nitric acid is a very effective reagent in the primary stages of the oxidation of coals and various forms of carbon, but to complete the oxidation to benzenecarboxylic acids it has been found advantageous to follow the primary nitric acid oxidation with a secondary one in alkaline medium.[2] The formation of stable lactone rings in the acidic stage would account for such behavior. This lactone of the biphenyl hydroxy acid is relatively insoluble in aqueous sodium carbonate and this fact suggests an explanation for the lower oxidation rates of coal in sodium carbonate compared with sodium hydroxide solutions. The highest methoxyl values for "regenerated humic acids" are obtained by the Waliaschko[3] method, where the compound is dissolved in an excess of alcoholic potash before reaction with dimethyl sulfate. One would expect very complete opening of lactone rings under such circumstances. The esters of acids from oxidation of coal have been shown to form adducts with stannic chloride in dilute pentane solutions. This lactone forms such an adduct under identical experimental conditions.

It has been reported[4] that the rate of reaction of ozone on coal is markedly affected by the presence of water and that the action of this oxidizing agent on "regenerated humic acids" is greatly accelerated if the acids have been previously treated with boiling aqueous alkali. These facts point to a hydrolytic step in the reaction mechanism.

It is well established that the reaction of steam or water with carbon is greatly accelerated by the presence of alkalies; the opening of peripheral lactone rings could be responsible for the effect. It is possible that surface oxygen complexes, such as the C_xO_y of Rhead and Wheeler,[5] consist in part of lactone rings in peripheral positions. That such lactones can be produced by gas phase oxidation has been demonstrated recently by Brooks[6] who obtained this identical lactone by the air oxidation of phenanthrene in a fluidized catalyst bed at 370°.

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COAL RESEARCH LABORATORY JACOB ENTEL
CARNEGIE INSTITUTE OF TECHNOLOGY CLARENCE H. RUOF
PITTSBURGH, PENNSYLVANIA H. C. HOWARD

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REACTIONS OF ALLYL ALCOHOL-1-C^{14}

Sir:

Although an allylic rearrangement would be expected to occur when allyl alcohol is transformed into an allyl halide under certain experimental conditions, the extent to which it takes place has not been determined. In the present work allyl alcohol-1-C^{14} was converted to radioactive allyl chloride and allyl bromide by different methods, the starting material and final products degraded with ozone, and the amount of rearrangement determined from the specific activity of the formaldehyde-C^{14}.

By modification of the excellent method of Young and Lane[1] carbon-14 labeled allyl bromide was prepared from allyl alcohol-1-C^{14}, phosphorus tribromide, and pyridine at −80°. Upon degradation with ozone, the per cent. rearrangement to allyl bromide-3-C^{14} was found to be 46%.

Radioactive allyl chloride was made from allyl alcohol-1-C^{14} and thionyl chloride by the method of Meisenheimer and Link[2] and the amount of rearrangement to allyl chloride-3-C^{14} was 51%.

Finally, the tosylate of allyl alcohol-1-C^{14} was treated with sodium bromide in a suitable solvent and only allyl bromide-1-C^{14} resulted indicating no rearrangement.

Further study is in progress with allyl alcohol-1-C^{14} and allyl bromide-1-C^{14} to determine if in those cases where rearrangement occurs a unimolecular process of replacement involving the formation of a resonating cation is the predominant mechanism.

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DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF ILLINOIS ROBERT F. NYSTROM
URBANA, ILLINOIS JOHN C. LEAK

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ENZYMATIC SYNTHESIS OF D-GLUTAMINE AND RELATED HYDROXAMIC ACIDS

Sir:

The mechanism of the enzymatic interaction of ATP,[1] L-glutamate, and ammonia, yielding ADP, L-glutamine, and inorganic phosphate has been of interest since the reaction was first described by Speck[2] and by Elliott.[3] Elliott,[4] using a highly purified enzyme from peas, was unable to separate glutamine synthesis from glutamotransferase ac-

(1) Abbreviations employed: ATP = adenosine triphosphate, ADP = adenosine diphosphate, tris = tris-(hydroxymethyl)-aminomethane.

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tivity.[5,6] Although it appears that glutamine synthesis probably occurs by a stepwise reaction, there is no clear evidence for an intermediate. Recently, Black and Gray[7] reported evidence for β-L-aspartyl phosphate as the product of the enzymatic reaction of L-aspartate and ATP, a finding which renews interest in the possibility of a similar intermediate in glutamine synthesis.

We now report the enzymatic synthesis of hydroxamic acids from D-glutamate, and from both isomers of α-aminoadipate, using the enzyme obtained from peas,[4] as well as enzymes from pigeon liver[2] and sheep brain.[3] When ammonia was substituted for hydroxylamine, D-glutamine was formed, but at less than half the rate observed for D-glutamohydroxamic formation. There was no detectable amide formation from α-aminoadipate under the conditions employed (Table I).

TABLE I

Units—μM formed/mg. enzyme N. Digests contained 50 μM $MgCl_2$, 100 μM neutralized hydroxylamine hydrochloride or NH_4Cl, 50 μM glutamic acid or α-aminoadipic acid neutralized with tris, 25 μM β-mercaptoethanol, 10 μM sodium ATP, 50 μM imidazole buffer, *p*H 7.0. Incubated at 37° for 15 to 40 minutes, with sufficient pea enzyme to effect the synthesis of 2 to 4 μM of hydroxamic acid or amide with active substrates. Final volume, 1.0 ml.; *p*H 7.0. Values corrected by subtraction of blanks. The isomers of aspartic acid are not appreciably active in this system.

Substrate	Hydroxamic acid formed[8] (units/hour)	Amide formed, expressed as phosphate liberated[9] (units/hour)
L-Glutamate	407	428
D-Glutamate	385	171
L-α-Aminoadipate	19.8	0
D-α-Aminoadipate	24.6	0

The D-glutamate[10] employed in these experiments was obtained from the racemate by enzymatic resolution[11] or by the action of *Cl. welchii* decarboxylase.[12] The D-glutamine formed enzymatically was isolated in crystalline form ($[\alpha]^{26}D$ −6.5°), and was identified by paper chromatography, and by its failure to yield carbon dioxide with *Cl. welchii* decarboxylase. Both isomers of glutamine are deamidated by this preparation, but only L-glutamine yields carbon dioxide.

The fact that hydroxamic acids are formed from L- and D-glutamate at similar rates, while D-glutamine is formed considerably less rapidly, suggests the possibility of an initial activation of the glutamate which is of low optical specificity, followed by a more specific reaction with ammonia which becomes rate limiting in the case of D-glutamate. Such a limitation is not noted with hydroxylamine, which is known to react non-enzymatically with acyl phosphates, thiolesters, and certain other compounds. The apparent failure to synthesize the amides of the α-aminoadipate isomers is compatible with the view that the reaction of ammonia with an intermediate is relatively specific. On the other hand, racemic α-methylglutamic acid reacts in this system with both hydroxylamine and ammonia.[13,14]

It is of interest that study of the transferase reaction indicates a high degree of specificity (Table II).

TABLE II

For units, see Table I. Digests contained 50 μM $MgCl_2$, 1 μM sodium ADP, 50 μM glutamine or α-aminoadipamic acid, 100 μM neutralized hydroxylamine hydrochloride, 20 μM β-mercaptoethanol, 5 μM phosphate buffer *p*H 6.6. Incubated at 37° for 15 to 30 minutes, with sufficient pea enzyme to effect the formation of 0.5 to 1.0 μM of hydroxamic acid. Final volume, 1.0 ml.; *p*H 6.5. Values corrected by subtraction of blanks.

Substrate	Hydroxamic acid formed[8] (units/hour)
L-Glutamine	286
D-Glutamine[15]	3.93
L-α-Aminoadipamic acid[15]	0

While these findings might be interpreted as indirect evidence for an intermediate acyl phosphate similar to that described by Black and Gray,[7] we have been unable to obtain any evidence for a free phosphorylated product of the reaction of ATP and the glutamate or aminoadipate isomers.

The authors wish to thank Dr. Jesse P. Greenstein for generous samples of the isomers of glutamic and α-aminoadipic[16] acids.

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NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH — LEON LEVINTOW
BETHESDA, MARYLAND — ALTON MEISTER

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THE SYNTHESIS OF UROPORPHYRIN I

Sir:

The structure proposed for uroporphyrin I by Hans Fischer[1] has been confirmed by synthesis.

The Pyrrole A[2] was brominated to give the crystalline methene B which, when fused with methylsuccinic acid for six hours at 118° *cf* [3], gave porphin-1,3,5,7-tetraacetic acid-2,4,6,8-tetrapropionic acid as the octamethyl ester m.p. 290–292°[4] (5.7%). Analysis gave no indication of partial decarboxylation [Calcd. for $C_{48}H_{54}O_{16}N_4$: C, 61.14; H, 5.77; N, 5.94; OCH_3, 26.33; C–CH_3, 0.0. Found: C, 61.00; H, 5.85; N, 5.76; OCH_3, 26.38; C-CH_3, 0.0.] In analogous cases, this method has given type I porphyrins exclusively. Here, the type was confirmed by partial decarboxylation to coproporphyrin I[5] obtained as the tetramethyl ester m.p.

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