solid, m.p. 192–204°, was 1.86 g. (57%). Two crystallizations from ethanol and treatment with activated charcoal gave 1.22 g. (38%) of colorless needles melting at 214–216° dec., $[\alpha]_D^{26}$ +118° (water, *c* 0.2). Spectral properties (water): At *p*H 6, ϵ_{max} (262 mμ) 10,100; ϵ_{min} (230 mμ) 2020. At *p*H 2, ϵ_{max} (260 mμ) 10,000; ϵ_{min} (229 mμ) 2120.

Anal. Calcd. for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.39; N, 17.28. Found: C, 44.58; H, 5.26; N, 16.92.

1-(3′-Acetamido-3′-deoxy-β-D-arabino-furanosyl)uracil (VII). A mixture of 0.60 g. (0.0025 mole) of V in 125 ml. of methanol was warmed until the solution was complete. After cooling to 20°, 0.27 ml. (0.0029 mole) of acetic anhydride was added. The solution was stirred at room temperature for 5 hr., then taken to dryness *in vacuo* (bath below 25°). The residue was triturated with ether and filtered. The yield of colorless prisms, m.p. 235–238°, $[\alpha]_D^{27}$ +123° (water, *c* 0.4), was 0.67 g. (96%).

Anal. Calcd. for $C_{11}H_{15}N_3O_6$: C, 46.31; H, 5.30; N, 14.73. Found: C, 46.45; H, 5.36; N, 14.58.

1-(3′-Acetamido-3′-deoxy-2′,5′-di-O-mesyl-β-D-arabinosyl)-uracil (VIII). A solution of 0.64 g. (0.0023 mole) of VII in 7 ml. of dry pyridine was allowed to react with 0.81 g. (0.0071 mole) of methylsulfonyl chloride at 0–5° for 17 hr. and at room temperature for 40 min. A small amount of the reaction mixture gave no precipitate upon addition of water. The addition of a mixture of 25 ml. of ether and 10 ml. of petroleum ether (b.p. 30–60°) to the main batch caused the precipitation of an amber colored gum. This was triturated with cold water (10 ml.), and a pink crystalline solid was collected. Crystallization from ethanol (20 ml.) gave 0.38 g. (38%) of colorless prisms melting at 129–135°. Two crystallizations from ethanol gave prisms melting at 133–150°, $[\alpha]_D^{25}$ +126° (water, *c* 0.2).

Anal. Calcd. for $C_{13}H_{19}N_3O_{10}S_2$: C, 35.37; H, 4.34; N, 9.52; S, 14.53. Found: C, 35.43; H, 4.19; N, 9.55; S, 14.39.

1-(3′-Amino-3′-deoxy-β-D-ribo-furanosyl)uracil (*3′-amino-3′-deoxyuridine*, VI). A mixture of 0.30 g. (0.00068 mole) of VIII, 0.30 g. (0.0037 mole) of sodium acetate, and 0.30 ml. of water was refluxed in 5.0 ml. of Methyl Cellosolve for 18 hr. The resulting dark colored mixture was taken to dryness *in vacuo*. The addition, followed by distillation *in vacuo*, first of ethanol, then of benzene, removed all water and solvent. The residue was dissolved in 5 ml. of pyridine and allowed to react for 17 hr. with 1.3 g. of acetic anhydride at room temperature.[29] To the reaction mixture was added 30 ml. of ether–petroleum ether (1:1) to precipitate out all nucleoside material. The supernatant was decanted, and the residue washed with ether–petroleum ether and dried. The water-soluble residue was heated on a steam bath for 80 min. with 4 ml. of sodium hydroxide (0.5*N*) and passed onto a column containing 10 ml. of Dowex 50 (H^+ form). The column was washed well with water, eluting a small amount of nonbasic material. The aminonucleoside was then eluted using ammonium hydroxide (0.5*N*).

The fraction containing ultraviolet-absorbing material was taken to dryness *in vacuo*. The residue was triturated with ether and filtered. The yield of a colorless amorphous solid, m.p. 120–130°, was about 0.02 g. (12%). Crystallization from ethanol gave a nearly quantitative recovery of minute colorless prisms. The sample melted at 183–185° dec. and had an optical rotation, $[\alpha]_D^{25}$ +67° (water, *c* 0.7).[30] A mixture of this sample and a mixture of an authentic sample of 3′-amino-3′-deoxyuridine,[25] m.p. 184–186°, melted at 183–186°. The infrared spectra (potassium bromide disks) of the two samples (both crystallized from ethanol) were identical in every respect. The probability that 3′-amino-3′-deoxyuridine may exist in two different crystalline forms with decidedly different infrared spectral curves is discussed earlier.

Acknowledgment. The authors wish to express their appreciation to Dr. George B. Brown for helpful suggestions and continued interest.

NEW YORK 21, N. Y.

(29) This step was undertaken in the hope of isolating a water-insoluble intermediate. Unfortunately, the acetylated product entered solution upon the addition of water.

(30) Kissman and Weiss[21] report a melting point of 183–184° and an optical rotation, $[\alpha]_D^{25}$ (water, *c* 1.0), of +67°.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE]

Synthesis of 1-β-D-Ribofuranosylimidazole-4(or 5)-acetonitrile, 1-β-D-Ribofuranosylimidazole-4(or 5)-acetic Acid, and 4(or 5)-(2-Aminoethyl)-1-β-D-ribofuranosylimidazole

HUGO BAUER

Received August 2, 1961

The synthesis of 1-β-D-ribofuranosylimidazole-4(or 5)-acetonitrile by condensation of the mercuric chloride complex of imidazoleacetonitrile with 2,3,5-tri-*O*-benzoyl-D-ribosyl bromide and subsequent debenzoylation is described. 1-β-D-Ribofuranosylimidazole-4(or 5)-acetic acid was obtained by the hydrolysis of the cyano group; 4(or 5)-(2-aminoethyl)-1-β-D-ribofuranosylimidazole (histamine ribose) by catalytic reduction of the cyano group.

From the urine of rats which had received injections of histamine or of imidazoleacetic acid, a compound was isolated by H. Tabor and O. Hayaishi, and by Karjala,[1] which was characterized as a riboside of imidazoleacetic acid (VI). This *in vivo* conversion prompted its chemical synthesis which was achieved simultaneously by the author[2] and by J. Baddiley *et al.*[3] This paper

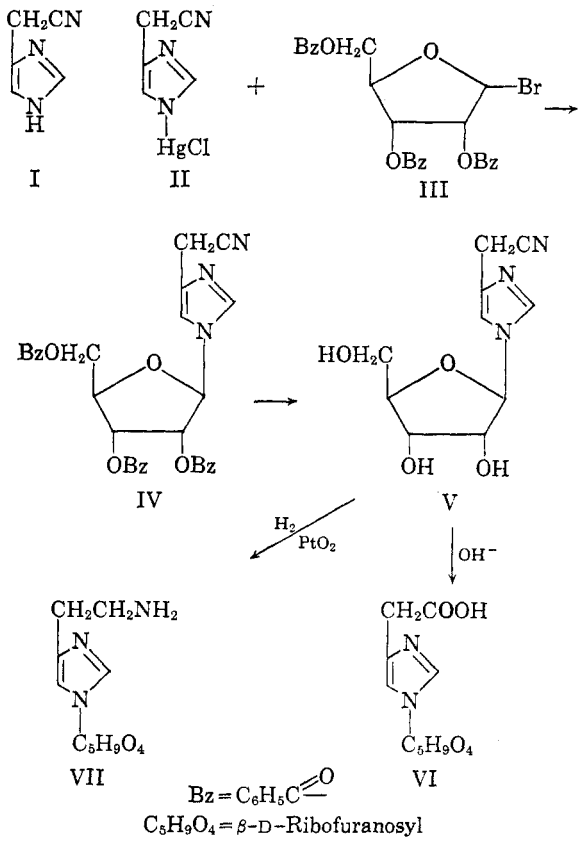
(1) H. Tabor, *Pharmacol. Rev.*, **6**, 331 (1954); H. Tabor and O. Hayaishi, *J. Am. Chem. Soc.*, **77**, 505 (1955); S. A. Karjala, *J. Am. Chem. Soc.*, **77**, 504 (1955).

(2) H. Bauer, *Biochim. et Biophys. Acta*, **30**, 219 (1958). Note change in nomenclature: Imidazoleacetic acid riboside to 1-β-D-ribofuranosylimidazole-4(or 5)acetic acid and cyanomethylimidazole to imidazoleacetonitrile.

(3) J. Baddiley, J. C. Buchanan, D. H. Hayes, and P. A. Smith, *J. Chem. Soc.*, 3743 (1958).

presents details of the synthesis of the β-D-ribofuranoside of imidazoleacetic acid, as well as of the related imidazoleacetonitrile and histamine. Syntheses of these compounds have not previously been described, although nucleosides of benzimidazoles[4] and of 5-amino-4-imidazolecarboxamide[5] have been prepared by several authors.

For the synthesis of the ribofuranosides of imidazoleacetic acid and of histamine, imidazole-4-(or 5)acetonitrile was used as a starting material. This was conveniently prepared by reacting histidine with sodium hypochlorite.[6] For the synthesis of nucleosides the procedure of Davoll and Lowy,[7] who introduced the chloromercury derivatives of the bases for condensation with acetylglycosyl halides, was adopted. The addition of Celite[8] to the reaction mixture greatly facilitated the condensation. Instead of using an acetylated glycosyl halide, however, a benzoylated glycosyl halide[9] was employed. Thus, by condensation of the mercuric chloride complex of imidazoleacetonitrile (II) with 2,3,5-tri-*O*-benzoyl-D-ribosyl bromide (III) in boiling xylene solution and subsequent debenzoylation with barium methoxide the riboside of imidazoleacetonitrile (V) was formed. It was possible to obtain V in an approximately pure state as an amorphous solid. During the purification of this compound by ion exchange chromatography, it was shown that the cyano group was unstable towards mineral acids. However, working at +5° was sufficient to suppress hydrolysis of the cyano group during elution with 2*N* sulfuric acid.



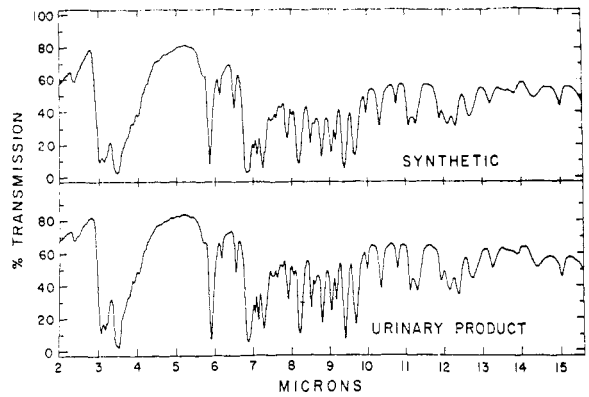


Fig. 1. Infrared spectra of urinary and synthetic 1-β-D-ribofuranosyl-4(or 5)acetic acid hydrochloride.

Hydrolysis of the cyano group of V with aqueous barium hydroxide yielded the riboside of imidazoleacetic acid VI.

This synthesis does not indicate which of the two nitrogen atoms of the imidazole ring is the site of the substitution by ribose. It may be designated as 1-β-D-ribofuranosylimidazole-4(or 5)acetic acid. By analogy with comparable reactions it is assumed that the configuration in this case is *trans* or β.[10] The identity of VI with the product isolated from urine was proved by melting point and mixed melting point, optical rotations, and infrared spectra (Fig. 1).

The isolation of V was not necessary when it was used as an intermediate for the preparation of imidazoleacetic acid riboside VI or of histamine riboside VII.

The catalytic reduction of the cyano group[11] in the presence of platinum oxide was applied to imidazoleacetonitrile in order to obtain histamine. This procedure worked also in the preparation of the histamine riboside VII by reducing V.

(4) N. G. Brink, F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill, and K. Folkers, *J. Am. Chem. Soc.*, **72**, 1866 (1950); J. Davoll and G. B. Brown, *J. Am. Chem. Soc.*, **73**, 5781 (1951); F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill, J. B. Lavigne, and K. Folkers, *J. Am. Chem. Soc.*, **74**, 4521 (1952); H. M. Kissman, R. G. Child, and M. J. Weiss, *J. Am. Chem. Soc.*, **79**, 1185 (1957); H. M. Kissman and M. J. Weiss, *J. Am. Chem. Soc.*, **80**, 5559 (1958).

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EXPERIMENTAL

Mercury complex of imidazoleacetonitrile II. Imidazoleacetonitrile (I) (2.14 g., 0.02 mole) and sodium carbonate (2.12 g., 0.02 mole) were dissolved in 150 ml. of hot water, followed by the addition of 3 g. of Celite.[8] A solution of mercuric chloride (5.43 g., 0.02 mole) in 150 ml. of hot water was then added with stirring. The white precipitate obtained was centrifuged, washed twice with water, and dried in the vacuum oven at 70°. The finely powdered material was freed of traces of water by azeotropic distillation with xylene; its suspension in about 150 ml. of xylene was used in the following condensation reaction.

Mercury complex of 1-(2,3,5-tri-O-benzoyl-β-D-ribosyl)-imidazole-4-(or 5)acetonitrile. 2,3,5-Tri-*O*-benzoyl-D-ribosyl-bromide (III) was prepared according to the procedure of Ness and Fletcher[9] from 9 g. of 2,3,5-tri-*O*-benzoyl-β-D-ribose[12]; the bromide obtained was dissolved in about 100 ml. of xylene and mixed with the xylene suspension of the mercury complex II. The reaction mixture was gently refluxed with stirring for 3 hr. and then filtered from the black residue. The xylene was removed by distillation under diminished pressure to leave a light brown oil "A" (about 10 g.).

Usually this oil was used for the later preparations without further purification. In one run however the oil "A" was further purified by extracting the ether-soluble materials which were discarded, and dissolving the ether insoluble residue (5 g.) in hot ethanol. Upon cooling, a dark resin deposited which was discarded. The supernatant was almost colorless and solidified to a gelatinous mass upon cooling and addition of petroleum ether. The yield was 1.6 g., the m.p. 95°.

Anal. Calcd. for $(C_{31}H_{25}N_3O_7)_2 \cdot HgCl_2 \cdot C_2H_5OH$: C, 54.11; H, 3.97; N, 5.92; Hg, 14.12; Cl, 4.99. Found: C, 53.51; H, 3.70; N, 5.77; Hg, 14.04; Cl, 5.00.

1-(2,3,5-Tri-O-benzoyl-β-D-ribosyl)imidazole-4(or 5)acetonitrile hydrochloride (IV). Oil "A" was freed of mercury by shaking its solution in dichloromethane with 30% potassium iodide solution. After evaporating the dichloromethane, the resulting oil was dissolved in ethanol and precipitated by addition of petroleum ether in three fractions. The middle fraction was a gum which solidified in the desiccator to a colorless material. After drying in the vacuum oven at 45°, it softened at 90°.

Anal. Calcd. for $(C_{31}H_{25}N_3O_7 \cdot HCl)_2 \cdot 3\ C_2H_5OH$: C, 62.14; H, 5.37; N, 6.40; Cl, 5.40. Found: C, 62.19; H, 4.81; N, 6.13; Cl, 5.38.

Debenzoylation of 1-(2,3,5-tri-O-benzoyl-β-D-ribosyl)imidazole-4(or 5)acetonitrile. For the following preparations 10 g. of the oil "A" was freed of mercury by shaking its solution in dichloromethane with 30% potassium iodide solution. The dichloromethane solution was dried over magnesium sulfate, the solvent removed, and the resulting oil dissolved in 25 ml. of methanol. An approximately 2*N* barium methoxide solution (10 ml.) was added. A thick precipitate of a barium salt formed. After standing overnight at room temperature, water and 2*N* sulfuric acid were added until the solution was just acid to Congo Red paper. The barium sulfate was centrifuged off, the supernatant liquor was extracted with ether and freed from volatile materials under diminished pressure. The resulting aqueous solution "B" was used for the preparation of the three ribosides V, VI, and VII.

1-β-D-Ribofuranosylimidazole-4(or 5)acetonitrile (V). Solution "B" was adsorbed on Dowex 50 (H-form, 8% cross-linked, 100–200 mesh, column 2.5 × 25 cm.) in the cold room at +5° and eluted with 1 l. of 2*N* sulfuric acid. The sulfuric acid was removed as barium sulfate; the neutral solution was made slightly alkaline with a few drops of 10% ammonium hydroxide and evaporated under diminished pressure at 30°. The residue was dissolved in water, decolorized by stirring with Norit at room temperature, and again evaporated to yield a colorless gum which solidified in the desiccator (1.7 g.). It was dissolved in absolute ethanol and freed from insoluble salts by filtration. A clear gum was obtained after evaporation. This material was contaminated by the riboside of imidazoleacetic acid which was largely removed by dissolving in hot absolute ethanol and cooling. The acid separated in fine flakes and was filtered off. The ethanolic filtrate was evaporated in the desiccator; the residue was washed with acetone and dried again. The solid obtained was deliquescent and contained one mole of water.

Anal. Calcd. for $C_{10}H_{13}N_3O_4 \cdot H_2O$: C, 46.69; H, 5.88; N, 16.34. Found: C, 46.73; H, 5.59; N, 15.63.

The low nitrogen found indicated a slight hydrolysis of the cyano group.

The ribose content of this riboside was determined by titration with sodium periodate, using the ultraviolet absorption of periodate as the indicator.[13] It reacted with one equivalent of periodate.

The cyano group is not stable in presence of mineral acids. Tests of imidazoleacetonitrile showed that 2*N* hydrochloric acid hydrolyzes the cyano group to the acetic acid group at room temperature after standing for 24 hr. Besides the formation of imidazoleacetic acid, the chromatogram in 1-propanol, 28% ammonium hydroxide, water (75 : 1.5 : 23.5) showed the presence of another product, probably imidazoleacetamide. No hydrolysis occurred at a temperature of about +5°, so that elution of the imidazoleacetonitrile riboside with mineral acid could be effected by working in the cold room.

1-β-D-Ribofuranosylimidazole-4(or 5)acetic acid hydrochloride (VI). *Hydrolysis of the cyano group.* To a solution "B," containing the riboside of imidazoleacetonitrile, a solution of 15 g. of barium hydroxide in 150 ml. of water was added. The mixture was boiled for 2 hr., until no more ammonia was evolved. Barium was removed with 2*N* sulfuric acid, the filtrate from barium sulfate was decolorized with Norit and adsorbed on Dowex-1-acetate (200–400 mesh, 2.5 × 25 cm.). The first fractions obtained by gradient elution with 3*N* acetic acid contained free imidazole acetic acid, as detected by coupling with diazotized *p*-nitroaniline (modified Pauly[14] test). The following noncoupling fractions were combined, acidified with 2*N* hydrochloric acid and evaporated. The white crystals obtained (1.5 g.) were recrystallized from water-acetone and melted at 185°. Over-all yield: starting from 2.14 g. (0.02 mole) of imidazoleacetonitrile, 1.5 g. (0.0051 mole) of 1.9 g. (0.0064 mole) of imidazoleacetic acid riboside hydrochloride corresponding to 25% to 32% were obtained.

Determination of the ammonia evolved by the hydrolysis of the cyano group with boiling 1*N* sodium hydroxide gave 94% of the calculated amount.

Anal. Calcd. for $C_{10}H_{13}N_2O_6Cl$: C, 40.75; H, 5.13; N, 9.51; Cl, 12.03. Found: C, 41.03; H, 5.37; N, 9.29; Cl, 12.24.

Comparison with the urinary product. The urinary product of m.p. 175° was recrystallized from water-acetone and gave a product with m.p. 185°. The mixed m.p. with the synthetic compound was 185°. Optical rotation of urinary product: $[\alpha]_D^{20}$ −36.6° (water, *c*, 1); −52.5° (methanol, *c*, 0.7). Synthetic product: $[\alpha]_D^{20}$ −37.0° (water, *c*, 1); −51.4° (methanol, *c*, 1.16). The infrared spectra (Nujol mull) of the urinary and synthetic nucleosides proved to be identical (Fig. 1).

Catalytic reduction of imidazoleacetonitrile to histamine. To a mixture of 20 ml. of ethanol and 2 ml. of concd. sulfuric acid,

(12) R. K. Ness, H. W. Diehl, and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, 76, 763 (1954).

(13) C. E. Crouthamel, H. V. Meek, D. S. Martin, and C. V. Banks, *J. Am. Chem. Soc.*, 71, 303 (1949); C. E. Crouthamel, A. M. Hayes, and D. S. Martin, *J. Am. Chem. Soc.*, 73, 82 (1951).

(14) H. Pauly, *Z. physiol. Chem.*, 42, 508 (1904); S. M. Rosenthal and H. Tabor, *J. Pharmacol. Exptl. Therap.*, 92, 425 (1948).

1.07 g. (0.01 mole) of imidazoleacetonitrile and 0.1 g. of platinum oxide were added. After shaking for 30 hr., 390 ml. of hydrogen was consumed (calcd. 445 ml.). The reaction mixture was adsorbed on Dowex 50, eluted with 4*N* hydrochloric acid, and the eluate evaporated under diminished pressure. The crude crystalline product obtained (1.0 g.) was recrystallized from ethanol and melted at 247–248°. When mixed with a sample of histamine dihydrochloride of m.p. 247–248°, the m.p. was 247–248°.

4(or 5)-(2-Aminoethyl)-1-β D-*ribofuranosylimidazole (histamine riboside) dihydrochloride* (VII). *Catalytic reduction of imidazoleacetonitrile riboside.* The concentrated aqueous solution "A" (about 50 ml.) was acidified with 0.5 ml. of concd. sulfuric acid and shaken with hydrogen with addition of 0.6 g. of platinum oxide in two portions. The amount of absorbed hydrogen varied between 360 and 450 ml. (calcd. 890 ml.). Hydrolysis of a sample of the crude reduced solution by heating with 2*N* hydrochloric acid at 150° and chromatography (solvent as before, Pauly[14] test) showed the presence of histamine and imidazoleacetic acid (formed by acid hydrolysis of the cyano group) in about equal amounts.

The reduced solution, after removing most of the sulfuric acid with barium hydroxide, was filtered through a column of Dowex 50 H^+ and eluted with 2*N* hydrochloric acid. Evaporation of a typical sample, which had absorbed 360 ml. of hydrogen, gave 2.36 g. of a crystalline product. The theoretical yield, calculated from hydrogen absorption, was 2.56 g. of histamine riboside dihydrochloride.

Recrystallization from water-ethanol gave colorless crystals of m.p. 174–175° (Kofler stage).They are soluble in water, sparingly soluble in ethanol, acetone, or ethyl acetate.

Anal. Calcd. for $C_{10}H_{17}N_3O_4 \cdot 2$ HCl: C, 37.99; H, 6.06; N, 13.29; Cl, 22.43. Found: C, 37.65; H, 6.09; N, 13.25; Cl, 22.30.

Acknowledgment. This work was supported by a grant from the National Science Foundation. It is a pleasure to thank Drs. H. Tabor and H. G. Fletcher, Jr., for helpful discussions; Dr. H. Tabor for urinary imidazoleacetic acid riboside; Drs. H. G. Fletcher, Jr., and R. K. Ness for a supply of tribenzoylribose; Dr. W. C. Alford and Mr. H. G. McCann and their staff for the microanalyses; and Mr. W. Jones for the infrared absorption spectra.

BETHESDA 14, MD.

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

Structure of Soil Humic Acid. II. Some Copper Oxide Oxidation Products[1-3]

GISELE GREENE AND CORNELIUS STEELINK

Received June 9, 1961

The oxidation of soil humic acid with aqueous alkaline cupric oxide produced a mixture of phenolic aldehydes and acids. A paper chromatographic analysis of the mixture revealed the presence of vanillin, *p*-hydroxybenzaldehyde, syringaldehyde, *p*-hydroxybenzoic acid, vanillic acid, 3,5-dihydroxybenzoic acid, and *m*-hydroxybenzoic acid. Samples of humic acid taken from three widely separated geographic regions all yielded the same products on degradation.

The co-occurrence of a resorcinol derivative (3,5-dihydroxybenzoic acid) with guaiacyl derivatives (vanillin, syringaldehyde, *etc.*) has not been previously reported in the studies of humic acid, and its presence cannot be rationalized on the basis of the commonly accepted theory that lignin is the precursor of soil humic acid. Many plant polyphenols and microbiological metabolites are structural derivatives of resorcinol, however, and occur in the zones of humification. The possible role of these substances in humic acid biosynthesis is discussed in the light of these experimental results.

Humic acid is a dark brown polymeric substance which occurs in the organic matter of soils and composts. It may contain 0–4% fixed nitrogen and be chelated to a variety of metallic ions in the soil. The presence of phenolic hydroxyl and carboxyl groups has been established, but little is known about the chemical structure of this acid.

Oxidative degradation[4-8] of humic acid yields a variety of phenols, phenolic aldehydes and phenolic acids, all of which can be obtained from lignin by the same chemical treatment. Although the bulk of the chemical evidence seems to favor the lignin-origin theory[9] for the biosynthesis of humic acid, recent work[2] in this laboratory indicates other possibilities. From a potassium hydroxide fusion of soil humic acid, we were able to isolate resorcinol, a phenol associated with breakdown products of plant polyphenols other than lignin, as well as with breakdown products of microbiological metabolites. To further investigate the nature of the phenolic degradation products, we decided to employ the mild oxidative technique of Pearl.[10a,b] The disadvantage of the potassium hydroxide fusion is that

(1) Presented at the 140th Meeting of the American Chemical Society, Chicago, Ill., September 7, 1961.

(2) The first paper in this series: J. W. Berry, A. Ho, H. E. Nordby, and C. Steelink, *Sci. Proc. Royal Dublin Soc.*, Series A, Vol. 1, 59–69 (1960).

(3) Abstracted from the master's thesis of Gisele Green, University of Arizona, 1961. This research was supported by the National Institutes of Health through Grant # RG-6058. Grateful acknowledgment is hereby made to the donors of this fund.

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