spectrum showing hydroxyl and tosylate bands absent and disappearance of characteristic spiroketal bands. A peak was present at 1686 cm.$^{-1}$, characteristic of —C=C—O—C— linkages.[18a,b]

20-Isodesoxydiosgenin (5-20β,22a,25D-Spirostene) (XIII).—A mixture of 2.5 g. desoxydiosgenin, 0.7 g. of pyridine hydrochloride and 12 ml. of acetic anhydride was refluxed for 6 hours. Following the usual ether work-up, the residue was refluxed 0.5 hour in 10% methanolic potassium hydroxide to give pseudodesoxydiosgenin. The latter was taken up in methanol and an equal volume of glacial acetic acid was added. After standing overnight, the usual ether work-up gave 0.6 g. of XIII, plates from methanol, m.p. 160–163°, $[\alpha]^{25}D$ dioxane −110°; infrared spectrum similar to X and XI plus additional unsaturation peak at 835 cm.$^{-1}$. *Anal.* Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.14; H, 10.64. Hydrochloric acid reflux of XIII gave desoxydiosgenin.

20-Isodesoxyyamogenin (5-20β,22b,25L-Spirostene) (XIV).—In the same manner as described under XIII, desoxyyamogenin was converted to XIV as plates from acetone, m.p. 184–186°, $[\alpha]^{25}D$ dioxane −12.3°, infrared spectrum similar to IX plus additional unsaturation peak at 838 cm.$^{-1}$. *Anal.* Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.39; H, 10.70. Treatment of XIV with hydrochloric acid gave desoxyyamogenin.

Acknowledgment.—The authors wish to thank R. F. Mininger for the optical rotations, K. Zbinden for carbon and hydrogen analyses, and C. R. Eddy and C. S. Fenske for the infrared data.

PHILADELPHIA 18, PENNA.

(18) (a) H. Rosenkrantz and M. Gut, *Helv. Chim. Acta*, **36**, 1000 (1953); (b) Hayden, *et al.*, ref. 14.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Studies in the Aminodeoxyinositol Series. III. Acetates of *myo*-Inosamine-2 with a Free Hydroxyl Group[1,2]

BY GEORGE I. DRUMMOND[3] AND LAURENS ANDERSON

RECEIVED AUGUST 22, 1955

From penta-O-acetyl-*myo*-inosamine-2 two isomeric N-acetyltetra-O-acetyl derivatives have been obtained by acetyl migration under mildly alkaline conditions. A series of sulfonyl esters were prepared for use in the characterization of these compounds. One of the isomers (A) has been shown to carry its free hydroxyl *ortho* to the acetylamino group. The position of the free hydroxyl in the other isomer (B) was not established.

For a program underway in this Laboratory, it was necessary to have an inosamine[4] (aminodeoxyinositol) with a single free hydroxyl group, *i.e.*, with the amino group and four of the five hydroxyls blocked. The preparation of two such N-acetyltetra-O-acetyl-*myo*-inosamines-2 is described in the present communication.

In paper I of this series,[5] it was shown analytically that when penta-O-acetyl-*myo*-inosamine-2 hydrochloride (I) is treated mildly with alkali, O→N acetyl migration takes place. These observations were extended and three procedures were found which yielded, on a preparative scale, a mixture of products containing a free hydroxyl group. The procedures were: (1) allowing the hydrochloride to stand in aqueous solution at room temperature for 24 hours after titration to *p*H 8 with sodium hydroxide; (2) treatment of the free base, penta-O-acetyl-*myo*-inosamine-2 (II), with aqueous pyridine at room temperature; and (3) treatment of the free base with water at 90°.

Two compounds were recovered from the migration mixtures by a simple solvent extraction. The elemental and functional group analyses of both compounds corresponded with those expected for an N-acetyltetra-O-acetylinosamine. They thus apparently differ only in the position of the free hydroxyl. If only O→N acetyl migration occurred, then only that compound carrying the free hydroxyl *ortho* to the amino group should be formed. The *meta* and *para* isomers could result, however, from a sequence of reactions involving O→O acetyl migration. A direct migration could also take place from the *para* position if the molecule existed in the boat conformation. The chair form has been suggested as the most stable conformation in the cyclohexane series,[6,7] but it is clear, from the fact that scyllitolcarboxylic acid forms a δ-lactone,[8] that an inositol can be brought into the boat form. Mechanisms therefore probably exist which could operate to leave a hydroxyl open in any of the three positions relative to the amino group. There are thus actually three possible isomeric N-acetyltetra-O-acetyl-*myo*-inosamines-2—two DL-pairs and one *meso* form. These are shown in the chart (formulas III, IV and V). The compounds isolated presumably represent two of these three possibilities. For convenience, the higher melting product is designated isomer A, and the lower melting one isomer B.

All three of the procedures mentioned above gave the same mixture of migration products, in which the ratio of isomer A to isomer B was about 1:5. It was found that when a sample of the free base II is melted in a capillary, it quickly resolidifies, and then has a second melting point, identical with the melting point of migrated isomer A. This observation suggested that migration might be brought about by heating, and that thermal migration might be used to prepare pure isomer A. Further investi-

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported by a research grant (G-3707) from the National Institutes of Health, Public Health Service.

(2) Paper II of this series: Helga Straube-Rieke, H. A. Lardy and L. Anderson, THIS JOURNAL, **75**, 694 (1953).

(3) Wisconsin Alumni Research Foundation unassigned Fellow, 1951–1953.

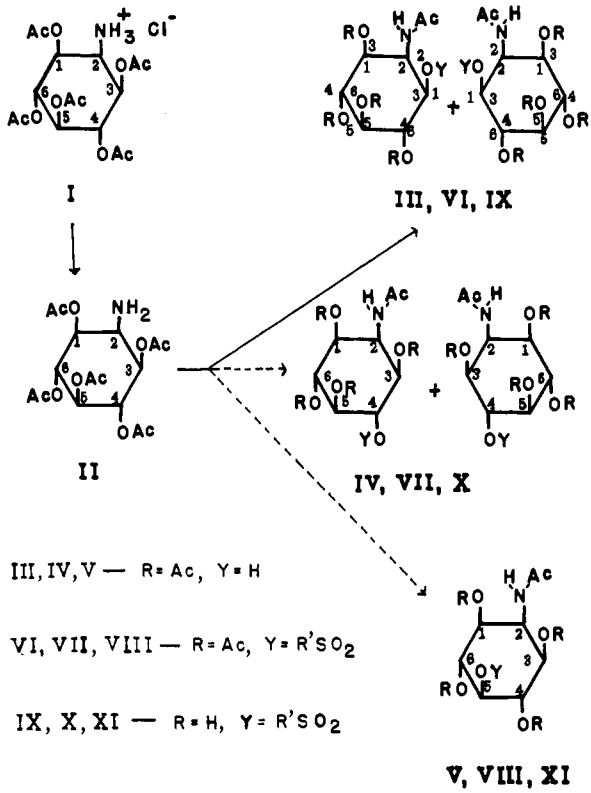
(4) The system proposed by H. G. Fletcher, Jr., L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951), is used for naming and numbering the compounds described in this paper.

(5) L. Anderson and H. A. Lardy, THIS JOURNAL, **72**, 3141 (1950).

(6) S. J. Angyal and J. Mills, *Revs. Pure and Appl. Chem.* (*Australia*), **2**, 185 (1952).

(7) H. D. Orloff, *Chem. Revs.*, **54**, 347 (1954).

(8) Th. Posternak and D. Reymond, *Helv. Chim. Acta*, **36**, 1370 (1953).



gation verified the thermal migration, but the products obtained by heating larger batches of II were mixtures.

The characterization of the isomeric migration products is theoretically easy. Sulfonylation of the free hydroxyl, followed by O-deacetylation, should yield an N-acetylinosamine O-sulfonate which could be analyzed by the periodate method. Since pyridine was shown to catalyze acetyl migration in aqueous solution, it became necessary to inquire whether further migration might occur during sulfonylation, which is carried out in dry pyridine. Some information on this point was obtained by studying the crude methanesulfonyl (mesyl) esters. It was found that the ratio of isomers in these products corresponded closely with the ratio of isomers in the starting material. Pure migrated isomer A yielded a single mesyl ester; slightly impure isomer B yielded a different mesyl ester, contaminated with a little of the first; and the crude migrated material yielded a mixture of the two mesyl esters in the approximate proportion of 1:5. It seems likely that, had migration occurred during sulfonylation, the mesyl esters from the pure or nearly pure isomers would have been mixtures approaching in composition the product from the crude migrated material.

In the search for derivatives which would behave well in the deacetylation step, a series of sulfonyl esters were made. Thus, in addition to the mesyl derivatives, the *p*-toluenesulfonyl (tosyl), *p*-bromobenzenesulfonyl (brosyl), and β-naphthalenesulfonyl (nasyl) esters of isomer B were prepared, and the tosyl and nasyl esters of isomer A.

Deacetylation, with ethanolic ammonia, of the mesyl ester of isomer A afforded a crystalline derivative which analyzed correctly for an N-acetyl-O-mesylinosamine. This derivative was eventually analyzed successfully with periodate. Isomer B, on the other hand, has so far proved refractory. The deacetylated mesyl and tosyl esters were sirups which could not be crystallized. The brosyl ester was desulfonylated, as well as deacetylated, by the ethanolic ammonia, as indicated by the isolation of N-acetyl-*myo*-inosamine-2 in good yield from the reaction mixture. In view of the fact that the nasyl ester of isomer A gave a product of low sulfur content, indicating partial desulfonylation, the deacetylation of nasyl B was not investigated.

In order to distinguish between N-acetylinosamine-O-sulfonates of the three possible structures IX, X and XI by periodate analysis, both the periodate consumption and the formic acid production must be measured. The theoretical values (moles per mole) are: periodate, *ortho* (IX), 3; *meta* (X), 2; *para* (XI), 2; formic acid, *ortho*, 2; *meta*, 1; *para*, 0. Both the crystalline mesyl derivative from isomer A and the sirupy one from isomer B were overoxidized at room temperature; *i.e.*, the periodate consumption and formate production did not level off at a fixed value. At 0°, the behavior of the sirup was not improved, but the oxidation of the crystalline material stopped cleanly when 3.0 moles of periodate had disappeared. The yield of formic acid was 2.1 moles. Isomer A therefore has the *ortho* structure III, and is DL-N-acetyl-1,4,5,6-tetra-O-acetyl-*myo*-inosamine-2. Isomer B must possess one of the remaining structures IV or V.

Experimental[9]

***myo*-Inosamine-2.**—Part of the *myo*-inosose-2 used as the starting material was kindly furnished by the Corn Products Refining Co. Additional quantities were prepared by the procedure of Posternak.[10] The method of Carter, *et al.*,[11] was used to convert the inosose to the oxime, which was catalytically hydrogenated essentially as described by Anderson and Lardy.[5] Quantities of the oxime up to 20 g. were conveniently reduced at atmospheric pressure in an apparatus employing a Vibro-Mixer[12] with hydrogenation stirrer. *myo*-Inosamine-2 was recovered by evaporating (vacuum) the filtered solution, dissolving the resulting sirup in a minimum of water, and making the solution slightly alkaline with concentrated potassium hydroxide. The precipitated amine was filtered off after 2 hours in the refrigerator and washed well with ice-water, then with ethanol, and dried.

Penta-O-acetyl-*myo*-inosamine-2 Hydrochloride (I).—*myo*-Inosamine-2 was converted to I essentially as described by Anderson and Lardy.[5]

Penta-O-acetyl-*myo*-inosamine-2 (II).—In a typical run 13 g. of the hydrochloride (I) was dissolved in 130 ml. of water at 40° and the solution chilled in an ice-bath. Sodium bicarbonate (2.6 g.) was dissolved in 30 ml. of water and added with stirring, and the solution kept in the ice-bath for 15 minutes. The precipitated free base was filtered, washed well with ice-water, and dried *in vacuo;* yield 10.3 g. (88%). The compound melted at 189° (bath temperature rise 8° per minute), then solidified and melted again

(9) All melting points were taken in capillary tubes. The thermometer used was standardized against an NBS calibrated Anschütz set. C, H and S analyses are by the Micro-Tech Laboratories, Skokie, Illinois. Nitrogen was determined by the semi-micro Kjeldahl technique. Acetyl analyses were carried out according to E. P. Clark, *Ind. Eng. Chem., Anal. Ed.*, **8**, 487 (1936), and **9**, 539 (1937).

(10) Th. Posternak, *Biochemical Preparations*, **2**, 57 (1952).

(11) H. E. Carter, *et al.*, *J. Biol. Chem.*, **174**, 415 (1948).

(12) The Vibro-Mixer (Fisher Scientific Co.) is a motor which vibrates a stirrer through a small vertical amplitude, rather than rotating it.

at 245–247°. For analysis, 200 mg. was recrystallized two times (rapidly) from 6 ml. of boiling ethanol.

Anal. Calcd. for $C_{16}H_{23}O_{10}N$ (389.35): C, 49.35; H, 5.95; N, 3.60; total acetyl, 55.28. Found: C, 49.36; H, 6.04; N, 3.62 (Van Slyke), 3.59 (Kjeldahl); total acetyl, 55.30.

Acetyl Migration (Analytical). a. By Titration to *p*H 8.—The hydrochloride I (2.45 g.) was dissolved in 50 ml. of water and 25 ml. of dioxane was added. The solution was then titrated to *p*H 8 with 0.1 *N* NaOH and water was added to exactly 250 ml. Aliquots (2.0 ml.) were removed periodically and analyzed for free amino nitrogen by the Van Slyke method. The results are shown in Fig. 1. The remaining solution was then evaporated to dryness *in vacuo* and the residue was washed free of chlorides, dried, and eventually fractionated.

b. **In Aqueous Pyridine.**—Two hundred mg. of the free base II was dissolved in 3 ml. of pyridine and water was added with swirling to exactly 10 ml. At various time intervals 1.0-ml. aliquots were removed and analyzed by the Van Slyke procedure. Figure 1 shows that the migration is considerably faster than by the first method.

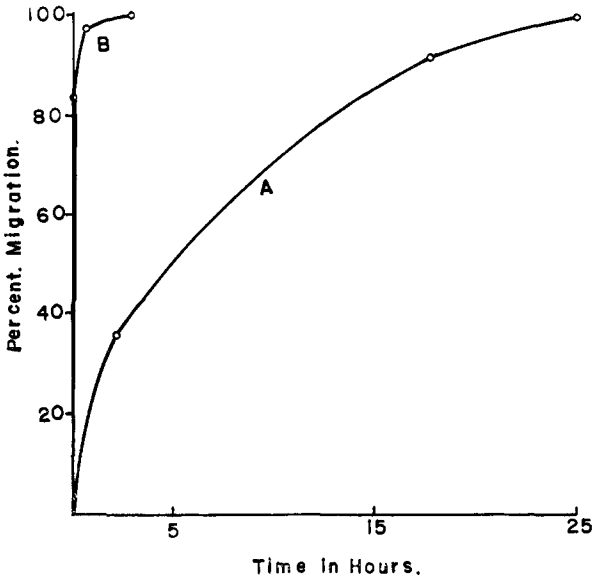


Fig. 1.—O→N acetyl migration in penta-O-acetyl-*myo*-inosamine-2: A, room temperature, initial *p*H 8; B, room temperature, in 30% pyridine.

c. **In Hot Water.**—The free base (659 mg.) was dissolved in 20 ml. of boiling water. After 1 hour at 90° the solution was cooled. A 0.6-ml. aliquot gave a negative Van Slyke.

d. **Thermal Migration.**—Two samples of the free base were held briefly above the melting point, then recrystallized and titrated electrometrically (Dr. R. M. Bock). Sample 1: 50 mg., 3 min. in bath at 200°, m.p. of product, 233–236°. Sample 2: 25 mg., 1.5 min. in bath at 250°, m.p. of product, 232–241°. (*Cf.* the melting points of the pure migrated isomers, below.) Neither sample had any free $-NH_2$. An elementary analysis of sample 1 showed the original proportions of C, H and N.

N-Acetyltetra-O-acetyl-*myo*-inosamines-2 (Isomers A and B) by Preparative Acetyl Migration.—For routine preparations, solutions of up to 10 g. of the free base were prepared in fifty volumes of 30% (v./v.) pyridine as described above and allowed to stand at room temperature for at least 3 hours (in some cases overnight) before an aliquot was removed and analyzed to confirm migration. The solvent was then evaporated in vacuum and the residue thoroughly dried.

In a typical fractionation, 5.2 g. of this material was finely triturated and stirred for 10 minutes with 30 ml. of a 3:7 benzene–acetone mixture. The insoluble residue was filtered off with light suction and washed with 10 ml. of the solvent. The filter cake was then slurried with an additional 10 ml. and filtered and washed as before. After a third extraction with 10 ml. of solvent, the residue consisted of pure isomer A (III), m.p. 245–247°, weight 1.3 g. The compound was recrystallized from 45 volumes of boiling ethanol.

The combined filtrates from the above extraction were evaporated to dryness and recrystallized several times from ethanol (*ca.* 20 volumes) to give isomer B (IV or V), m.p. 225–230°. The material could not be obtained entirely free of isomer A.

Anal. Calcd. for $C_{16}H_{23}O_{10}N$ (389.35): C, 49.35; H, 5.95; N, 3.60; total acetyl, 55.28. Found, Isomer A: C, 49.13; H, 6.00; N, 3.62; total acetyl, 55.16, 55.00. Found, Isomer B: C, 48.89; H, 5.96; N, 3.61, 3.56.

Mesyl Ester of Isomer A (DL-N-Acetyl-1,4,5,6-tetra-O-acetyl-3-O-mesyl-*myo*-inosamine-2) (VI, R′ = CH_3).—One gram of isomer A was dissolved in 10 ml. of pyridine and chilled in an ice-bath. Freshly distilled methanesulfonyl chloride (0.4 ml.) was then added and the mixture placed in the refrigerator. After 24 hours 2 ml. of water was added to decompose the mesyl chloride; one-half hour later 5 volumes of water was run in under stirring (ice-bath). The crystalline precipitate was filtered off, washed well with water and dried; wt. 1.0 g. (85%), m.p. 234–235°. The compound separates from acetone–water as long silky needles. After four recrystallizations from this solvent (8 ml. of acetone and 6 ml. of water were used the first time) and one recrystallization from boiling ethanol, the material (830 mg.) melted at 239°.

Of **Isomer B** (VII or VIII, R′ = CH_3).—One gram of isomer B (slight shrinking at 218°, m.p. 225–230°) when treated as above yielded 1.05 g. (87%) of crystalline mesyl ester melting at 205–206°. The material was dissolved in 15 ml. of boiling acetone, decolorized, and concentrated to 10 ml., and 5 ml. of water was added. After 7 hours at room temperature, the heavy prisms were filtered off and washed with 50% acetone. When dry they melted at 209–210°. The mother liquor upon concentration yielded 24 mg. of isomer A mesyl ester. Recrystallization of the main fraction from acetone–water raised the m.p. to 212–213° and the concentrated mother liquor again deposited isomer A mesyl ester (55 mg.). The main fraction was then recrystallized twice from boiling ethanol (75 volumes) to yield 750 mg. of heavy prisms melting at 213–214°.

Anal. Calcd. for $C_{17}H_{25}O_{12}NS$ (467.45): C, 43.68; H, 5.39; S, 6.86; N, 3.00; total acetyl, 46.04. Found, Isomer A: C, 43.54; H, 5.40; S, 6.76; N, 2.97; total acetyl, 48.20. Found, Isomer B: C, 43.11; H, 5.29; S, 6.63; N, 2.97; total acetyl, 46.05.

These two esters were also prepared from unfractionated migrated material. The mesyl ester of isomer B could be separated conveniently from the mixture by fractional crystallization from acetone–water by virtue of its low solubility in this solvent pair as compared with the mesyl ester of isomer A. However, the latter compound could be obtained pure only with difficulty by this method.

Additional Sulfonyl Esters.—The tosyl, brosyl and nasyl esters were prepared essentially as described by Bates, *et al.*[13] Some reaction mixtures were allowed to stand for 70 hours at room temperature. Considerable tar was formed in these reaction mixtures, with the result that yields were low, as compared with those of the mesyl esters, and the products were difficult to purify. The esters were recrystallized from acetone–water solutions and from large volumes of boiling ethanol.

a. **Tosyl Ester of Isomer A** (VI, R′ = *p*-$CH_3C_6H_4$), m.p. 214–216°. *Anal.* Calcd. for $C_{23}H_{29}O_{12}NS$ (543.54): C, 50.83; H, 5.38; N, 2.58. Found: C, 50.92; H, 5.67; N, 2.57, 2.58.

Of **Isomer B** (VII or VIII, R′ = *p*-$CH_3C_6H_4$), m.p. 207–209°. *Anal.* Found: C, 50.73; H, 5.51; N, 2.41, 2.40.

b. **Brosyl Ester of Isomer B** (VII or VIII, R′ = *p*-Br-C_6H_4), m.p. 226°. *Anal.* Calcd. for $C_{22}H_{26}O_{12}NSBr$ (608.43): C, 43.42; H, 4.31; N, 2.30. Found: C, 43.70; H, 4.53; N, 2.29, 2.31.

c. **Nasyl Ester of Isomer A** (VI, R′ = β-$C_{10}H_7$), m.p. 223–224°. *Anal.* Calcd. for $C_{26}H_{29}O_{12}NS$ (579.57): C, 53.88; H, 5.04; N, 2.42. Found: C, 53.83; H, 5.23; N, 2.41, 2.42.

Of **Isomer B** (VII or VIII, R′ = β-$C_{10}H_7$), m.p. 228–229°. *Anal.* Found: C, 53.74; H, 5.11; N, 2.43, 2.38.

(13) "Polarimetry, Saccharimetry and the Sugars," National Bureau of Standards Circular C440, Washington, D. C., 1942, p. 505.

DL-1(=3)-O-**Mesyl-N-acetyl-*myo*-inosamine-2** (IX, R′ = CH_3).—Two hundred mg. of the mesyl ester of isomer A was treated with 40 ml. of absolute ethanol saturated with ammonia at 4°. After 24 hours in the refrigerator the solvent was evacuated and the acetamide sublimed in vacuum (0.05–0.1 mm., bath temperature 90°). The clear sirup was crystallized from 4 ml. of methanol with a few drops of ether; yield 110 mg. (84%), m.p. 208° dec. Three further crystallizations from ethanol with a trace of water failed to alter the melting point.

Anal. Calcd. for $C_9H_{17}O_8NS$ (229.31): C, 36.11; H, 5.73; S, 10.71. Found: C, 36.20; H, 5.98; S, 10.76.

Periodate Analyses.—The crystalline deacetylated mesyl ester of isomer A and the corresponding sirupy ester of isomer B were analyzed by the method of Fleury and Lange.[14] In a typical run 0.5 mmole of the sample was dissolved in *ca.* 2 ml. of water and 22.5 ml. of 0.321 *M* sodium metaperiodate added at 4°. The solution was diluted to exactly 25 ml. with water, mixed, and placed in the refrigerator (4°). Initial molarity of sample, 0.02; of periodate, 0.28. At various time intervals 2.0-ml. aliquots were removed and discharged into a solution of 1.5 g. of $NaHCO_3$ in 25 ml. of water. Excess periodate was titrated by the arsenite method.

For formate analyses, similar aliquots were removed and mixed with several drops of ethylene glycol in 10-ml. erlenmeyer flasks. After one hour the solution was titrated to a methyl red end-point with 0.01 *N* NaOH. A blank was simultaneously prepared and titrated.

(14) P. Fleury and J. Lange, *J. pharm. chim.*, **17**, 196 (1933).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGY, THE UNIVERSITY OF ROCHESTER]

The Biosynthesis of Lignin: Evidence for the Participation of Celluloses as Sites for Oxidative Polymerization of Eugenol

BY S. M. SIEGEL

RECEIVED NOVEMBER 23, 1955

A study of the oxidation of eugenol by peroxidase–hydrogen peroxide has been extended in relation to previous work demonstrating formation of lignins from *p*-hydroxyphenylpropanes *via* a peroxidative pathway. Model systems supplied only with eugenol, enzyme and peroxide form water-insoluble oxidation products, which, however, are fully soluble in chloroform, and fail to yield lignin color tests. In the presence either of filter paper or a methylcellulose, however, chloroform-insoluble products are formed either in paper, if present, or as a coprecipitate with methylcellulose. Included in such products are ethanol-insoluble fractions giving positive color tests for lignin, in quantities of 12–25 mg./g. of paper. Relative to the amount of cellulose supplied, a methylcellulose solution supplies more polymerization sites than does filter paper. Experimental conditions and prospects for this line of investigation are considered.

Starting with the generally accepted concept that lignins contain, as fundamental units, one or more types of phenylpropanes,[1] the author has been able to establish a pathway for conversion of eugenol and other *p*-hydroxyphenylpropanes to lignins *via* the peroxidase–hydrogen peroxide system. Original experimentation showed that slices of peroxidase-containing tissues incubated with eugenol yield products capable of isolation by the usual methods of lignin work.[2] Among the identifying characteristics and properties of synthetic products studied were the following: solubility in a wide range of solvents, evaluated on a comparative basis together with natural lignins from oak, spruce, bean and other sources[3]; analysis which gave C 63%, H 7%, and OCH_3 15%, in good agreement with known lignins[4]; and ultraviolet absorption, which showed spectra of eugenol–synthetic lignin to be similar to a variety of lignins and essentially identical with those for spruce lignin.[4] The usual lignin color tests were also given by synthetic products.

The lignin-forming system described here may be contrasted with that described by Freudenberg, *et al.*,[5] and Freudenberg and Heel,[6] who started with coniferyl or hydroxycinnamyl alcohols rather than with eugenol. The present system possesses a higher rate of polymerization and is also completely dependent on the enzyme peroxidase. Freudenberg's system also involves strictly soluble materials. When eugenol is used as a substrate, lignin polymers are not formed by a cell- or particle-free system or in the presence of crystalline enzyme and peroxide alone.

The mechanism of enzymatic peroxidation, as suggested by such work as that of Westerfield and Lowe,[7] and Herzog and Meier,[8] and through the author's own observations probably entails formation of highly reactive intermediary semiquinones which may condense to yield a variety of reaction products, but which are incapable of forming the lignin polymer under the influence of enzyme alone. Resolution of the problem thus presented came with the observation that washed cell-wall material (with a substantial quantity of peroxidase tightly bound, however) could effect polymerization at nearly the same rate as that measured for entire tissue slices consisting largely of intact cells. This result was interpreted as meaning either that a specific polymerase resided in the wall, or that cellulose or other polysaccharides in the wall substance exerted an accessory catalytic effect on eugenol such that only those molecules in intimate association with such macromolecules were polymerized to lignins.

It is the object of this communication to describe the establishment of successful model systems, in which eugenol is peroxidatively converted into

(1) F. E. Brauns, "Chemistry of Lignin," Academic Press, Inc., New York, N. Y., 1952.

(2) S. M. Siegel, *Physiol. Plant*, **6**, 134 (1953).

(3) S. M. Siegel, *ibid.*, **7**, 41 (1954).

(4) S. M. Siegel, *ibid.*, **8**, 20 (1955).

(5) K. Freudenberg, H. Reznik, H. Boesenberg and D. Ranenack, *Chem. Ber.*, **85**, 641 (1952).

(6) K. Freudenberg and W. Heel, *ibid.*, **86**, 1955 (1953).

(7) W. W. Westerfield and C. J. Lowe, *J. Biol. Chem.*, **145**, 463 (1942).

(8) O. Herzog and A. Meier, *Z. physiol. Chem.*, **73**, 258 (1911).