were: oxalacetic acid 0.08, α-ketoglutaric acid 0.51, pyruvic acid 0.64, α-ketobutyric acid 0.76 and α-ketoisovaleric acid 0.83. In 95 parts *s*-butanol and 5 parts of propionic acid, saturated with water, the approximate R_F values, α-ketobutyric acid 0.40, α-ketoisovaleric acid 0.52 and α-keto-β-methyl-*n*-valeric acid 0.70, were observed.

The concentrations of the various ketoacids in a mixture could be determined by cutting small paper rectangles containing the isolated ketoacids from an untreated chromatogram, using a parallel semicarbazide treated strip as guide.[2] The paper rectangles were extracted with an aqueous solution of 2,4-dinitrophenylhydrazine hydrochloride and the 2,4-dinitrophenylhydrazones formed estimated colorimetrically.[3]

Similarly, the activities of the isolated ketoacids as growth factors and growth inhibitors could be determined by placing the untreated paper rectangles on minimal agar plates, seeded with a susceptible microörganism.[4]

With the aid of these methods the accumulation of α-ketoisovaleric acid and α-keto-β-methyl-*n*-valeric acid in unshaken culture fluids of a valine and isoleucine requiring mutant of *E. coli*, strain K12, could be demonstrated.

A detailed description of the method and its application to other problems will be forthcoming shortly.

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RECEIVED MARCH 20, 1950

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[2] Vischer and Chargaff, *J. Biol. Chem.*, **176**, 703 (1948).

[3] Friedemann and Haugen, *ibid.*, **147**, 415 (1943).

[4] Karnovsky and Johnson, *Anal. Chem.*, **21**, 1125 (1949).

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STUDIES ON LIGNIN AND RELATED PRODUCTS. III.[1] THE OXIDATION OF SULFITE SPENT LIQUOR WITH CUPRIC OXIDE UNDER PRESSURE[2]

Sir:

Recent isolations in good yield of several guaiacyl compounds other than vanillin from oxidations of lignosulfonate products with mercuric and silver oxides[1,3] prompted the reinvestigation of the oxidation of lignosulfonate materials with cupric oxide under pressure. This process was found in the past to result in high yields of vanillin (by analysis).[4] In the present studies a sulfite spent liquor of spruce origin, which had been fermented with *Torulopsis utilis*, was stirred with alkali and cupric hydroxide $[Cu(OH)_2]$ at elevated temperatures under pressure.

It soon became apparent that earlier yields of vanillin based upon precipitation with 2,4-dinitrophenylhydrazine were in error because this reagent was found to precipitate acetovanillone and other as yet unidentified guaiacyl compounds. Nevertheless, by changing reactant ratios, temperatures and times of reaction, conditions were found for obtaining vanillin in isolated yields at least as good as the analyzed yields reported earlier. In one experiment a mixture of 2800 g. of sulfite spent liquor (containing 72 g. or 0.34 mole of lignin), 180 g. (4.5 moles) of sodium hydroxide and 314 g. (3.2 moles) of cupric hydroxide was heated at 170° for five hours. In addition to vanillin, the following guaiacyl derivatives were produced by the process in substantial amounts: guaiacol, acetovanillone, vanillic acid, 5-carboxyvanillic acid, 5-carboxyvanillin, dehydrodivanillin and dehydrodivanillic acid.

The first four of these compounds were identified by methods noted before.[1,3] 5-Carboxyvanillin was isolated as white crystals melting at 251–252°; these gave the purple color with ferric chloride noted by Freudenberg and Klink[5] who recorded a melting point of 255° for the synthetic compound.

Anal. Calcd. for $C_9H_8O_5$: C, 55.09; H, 4.11; CH_3O, 15.82. Found: C, 54.99; H, 4.18; CH_3O, 15.79.

Dehydrodivanillin was isolated as colorless needles from phenyl cellosolve melting at 305° and not depressing a mixed melting point with authentic dehydrodivanillin.[6] Dehydrodivanillic acid was obtained as colorless needles from phenyl cellosolve melting at 299–300° and not depressing the melting point of a mixture with authentic dehydrodivanillic acid.[6]

The synthesis, degradation, derivatives and significance to the structure of lignin of these compounds will be discussed in forthcoming papers.

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[1] For Part II of this series, see THIS JOURNAL, **72**, 1427 (1950).

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

[3] Pearl, THIS JOURNAL, **71**, 2196 (1949).

[4] Pearl, *ibid.*, **64**, 1429 (1942).

[5] Freudenberg and Klink, *Ber.*, **73**, 1372 (1940).

[6] Elbs and Lerch, *J. prakt. Chem.*, **93**, 1 (1916).

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RECEIVED FEBRUARY 23, 1950

THE INCORPORATION *IN VIVO* OF THE ETHYL CARBON OF ETHIONINE INTO CREATINE AND CHOLINE OF RAT TISSUES

Sir:

The apparent specificity of the "labile methyl" group donors in the alleviation of the inhibition of growth by ethionine in the rat suggested the interesting possibility of involvement of the ethyl group of ethionine in the phenomenon of growth inhibition by way of replacement of the methyl group in the so-called transmethylation reactions.[1] Such a possibility implies de-ethylation of ethio-

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[1] J. A. Stekol and K. Weiss, *J. Biol. Chem.*, **179**, 1049 (1949).