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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(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).

(5) Public Health Service Research Fellow of the National Institutes of Health.

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.

(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).

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.

(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-

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

nine *in vivo* and the participation of the ethyl group directly or indirectly in the formation of choline, creatine, etc. The formation of such ethyl analogs *in vivo* at the expense of the normal methyl derivatives may at least in part account for the inhibition of growth of rats by ethionine. The data reported in this communication show that the ethyl group carbon of ethionine tagged with C-14 in the methylene carbon of the ethyl group appears in the creatine and choline of rat tissues. The radioactivity of the isolated choline was confined to the "trimethyl" amine moiety of the molecule.

Ten milligrams of C^{14}-ethionine (total activity 3.72×10^6 counts per minute) was injected intraperitoneally into each of two adult female rats of Wistar strain which were maintained on a complete casein diet. Six days later the food was withdrawn from the cages, the rats were fasted for 2 days, then sacrificed. From the entire carcass, minus the hide, choline was isolated as the reineckate and creatine as the creatinine potassium picrate. Choline reineckate was then converted to choline chloroplatinate. After the specific activities of the products were determined, choline chloroplatinate was degraded to "trimethyl" amine which was isolated as the hydrochloride. The activities of the compounds were determined directly on the weighed samples spread over an area of 5 sq. cm. in a G–M counter with a mica window of 1.8 mg. per sq. cm. The data are summarized in Table I.

TABLE I

	Specific activity, counts/min./millimole
C^{14}-Ethionine	6.06×10^7
Choline reineckate	7.83×10^3
Choline chloroplatinate	1.55×10^4
"Trimethyl" amine hydrochloride	8.9×10^3
Creatinine potassium picrate	2.36×10^3

The above data suggested the de-ethylation of ethionine in the rat. The removal of the ethyl group of ethionine may have proceeded *via* the formation of ethanol which, on oxidation, would eventually lead to the formation of acetic acid. To test this possibility, 10 mg. of C^{14}-ethionine was injected intraperitoneally into two adult rats which were maintained on a complete casein diet which was supplemented with 1% of S-benzyl-L-cysteine. We have shown previously that S-benzyl-L-cysteine is acetylated in the rat.[2] From pooled 3-day samples of urine of these rats analytically pure N-acetyl-S-benzyl-L-cysteine was isolated with the specific activity of 1.84×10^4 counts/minute/millimole. Complete data will be reported at a later date.

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FOX CHASE, PHILADELPHIA   KATHRYN WEISS
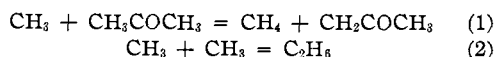   PENNSYLVANIA   SIDNEY WEISS
          RECEIVED MARCH 20, 1950

(2) J. A. Stekol, *ibid.*, **124**, 129 (1938).

THE REACTIONS OF METHYL RADICALS WITH HYDROCARBONS

Sir:

It has been shown[1] that the methane and ethane formed when acetone is photolyzed at 26 and 122° may be quantitatively accounted for by the reactions

$$CH_3 + CH_3COCH_3 = CH_4 + CH_2COCH_3 \quad (1)$$
$$CH_3 + CH_3 = C_2H_6 \quad (2)$$

We have investigated the photolysis between temperatures of 100 and 300°, at total pressures of from 3.7 to 100 mm. and with a thirty-fold variation of light intensity. Reactions (1) and (2) account quantitatively for all the methane and ethane produced. We found no indication that reaction (2) was subject to a third-body restriction or took place at the wall of the reaction vessel.

When a paraffin hydrocarbon is mixed with the acetone, methane is also produced by reaction (3)

$$CH_3 + RH = CH_4 + R \quad (3)$$
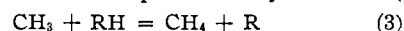
Then

$$R_{CH_4}/R^{1/2}C_2H_6 = k_1/k_2^{1/2} [Ac] + k_3/k_2^{1/2} [RH]$$

where k_1, k_2 and k_3 are the velocity constants of reactions (1), (2) and (3), R_{CH_4} and $R_{C_2H_6}$ are the rates of production of methane and ethane and [Ac] and [RH] are the concentrations of acetone and paraffin. The velocity constant of reaction (2) may be expressed as $k_2 = P_2 Z \exp(-E_2/RT)$. Because no absolute measure of the methyl radical concentration is available, the results are expressed in terms of $P_2^{1/2}$ and $1/2 E_2$. We have investigated the photolysis of acetone and deuterated acetone alone, acetone in the presence of six hydrocarbons and deuterated acetone in the presence of ethane. In each case excellent Arrhenius plots were obtained; we estimate the error in the relative activation energies to be less than ± 0.2 kcal. When the proportions of hydrocarbon and acetone were altered, the rates of formation of methane and ethane altered as predicted by the above mechanism. Our results are contained in Table I. The results show first that

TABLE I

Reactant	$E - 1/2E_2$ kcal.	$P/P_2^{1/2} \times 10^4$
Acetone	9.7	9.5
Deuterated acetone	10.3	...
Ethane	10.4	5.2
Neopentane	10.0	4.9
n-Butane	8.3	2.7
n-Pentane	8.1	2.5
n-Hexane	8.1	2.9
Isobutane	7.6	2.3
2,3-Dimethylbutane	6.9	1.7

in the series of the paraffin hydrocarbons the energy of activation for the abstraction of a primary hydrogen atom by a methyl radical is greater than that for the abstraction of a second-

(1) L. M. Dorfman and W. A. Noyes, Jr., *J. Chem. Phys.*, **16**, 557 (1948).
(2) W. A. Noyes, Jr., and L. M. Dorfman, *ibid.*, **16**, 788 (1948).