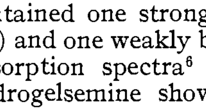
THE OXINDOLE NATURE OF GELSEMINE[1]

Sir:

It was claimed by Moore[2] that gelsemine contained a free hydroxyl group which could be acetylated with acetic anhydride. All attempts to prepare an O-acetylgelsemine have failed in our hands, and other workers[3] have been similarly unsuccessful. It can now be stated quite definitely that gelsemine does not contain a hydroxyl group. The base has been found to contain only one active hydrogen (Zerewitinow) and its infrared absorption spectrum to show a sharp absorption band at 3443 cm.$^{-1}$ indicative of an imino group. Since gelsemine is an indole alkaloid[4,5] and since the basic nitrogen is known to be tertiary, it follows that the one active hydrogen in the base must be attributed to the indole imino group.

The reaction of dihydrogelsemine with lithium aluminum hydride in dioxane gave rise to tetrahydrodesoxygelsemine, prisms, m. p. 138–139°. *Anal.* Calcd. for $C_{20}H_{26}ON_2$: C, 77.42; H, 8.39; N, 9.03; 1 act. H, 0.32. Found: C, 77.51, 77.34; H, 8.01, 8.21; N, 9.14; act. H, 0.33. This base forms a picrate, orange needles, m. p. 216–217° (dec.). *Anal.* Calcd. for $C_{20}H_{26}ON_2 \cdot C_6H_3O_7N_3$: C, 57.88; H, 5.38; N, 12.99. Found: C, 57.23, 57.40; H, 5.23, 5.19; N, 12.80. The new base contained one strongly basic nitrogen atom (pK 8.9) and one weakly basic (pK 3.4). The infrared absorption spectra[6] of both gelsemine and dihydrogelsemine showed an absorption band at 1720 cm.$^{-1}$ in the region of carbonyl absorption, while that of tetrahydrodesoxygelsemine showed no absorption in this region, but still contained an absorption band (3414 cm.$^{-1}$) indicative of a weakly associated imino group. Tetrahydrodesoxygelsemine contained one active hydrogen (Zerewitinow); it was acetylated by boiling acetic anhydride to an N-acetyl base, prisms, m. p. 131–132°. *Anal.* Calcd. for $C_{22}H_{28}O_2N_2$: C, 75.00; H, 7.95. Found: C, 75.81; H, 7.44. The acetyl derivative formed a crystalline acetate, plates, m. p. 182–186°. *Anal.* Calcd. for $C_{22}H_{28}O_2N_2 \cdot C_2H_4O_2$: C, 69.90; H, 7.77; N, 6.80. Found: C, 69.96, 69.70; H, 7.77, 7.58; N, 6.74, 6.77. The infrared absorption spectrum of the acetyl derivative showed no absorption band indicative of an imino group, but contained a carbonyl absorption band (1650 cm.$^{-1}$) obviously due to the acetyl group.

It can, therefore, be postulated that



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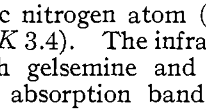

II

partial structure I is present in gelsemine and that it is the reduction of a carbonyl to a methylene group that gives rise to tetrahydrodesoxygelsemine in which partial structure II must be present. Oxindoles still containing a hydrogen atom in the 3-position are converted to indoles by the action of lithium aluminum hydride[7] and hence, the 3-position of the oxindole system in gelsemine must be assumed to be disubstituted. Indeed, the disubstituted 3,3-dimethyloxindole gave color changes with sulfuric acid and potassium dichromate very similar to those given by strychnine and gelsemine and its ultraviolet absorption curve was almost superposable on that of gelsemine. Furthermore, it has been ascertained that 3,3-dimethyloxindole is readily reduced by lithium aluminum hydride in dioxane solution to 3,3-dimethyldihydroindole (m. p. 34–34.5°). It is noteworthy that this result is contrary to the literature statement that N-unsubstituted oxindoles are virtually unaffected by lithium aluminum hydride.[7]

The partial structures I and II are further supported by the strychnidine-like properties of tetrahydrodesoxygelsemine. Like strychnine it must contain a nitrogen atom directly attached to an aromatic nucleus with a free and reactive para-position. When coupled with diazotized sulfanilic acid, it gave rise to a methyl orange which resembled in its indicator behavior that of sulfobenzoazostrychnidine.

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[1] Issued as N. R. C. Bull. No. 2082.

[2] Moore, *J. Chem. Soc.*, 1231 (1911).

[3] Forsyth, Marrian and Stevens, *ibid.*, 579 (1945).

[4] Marion, *Can. J. Research*, **21B**, 247 (1943).

[5] Witkop, THIS JOURNAL, **70**, 1424 (1948).

[6] All infrared spectra were determined in chloroform solution by Dr. R. Norman Jones.

[7] Julian and Printy, THIS JOURNAL, **71**, 3206 (1949).

CHEMISTRY DIVISION
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OTTAWA, CANADA
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RECEIVED FEBRUARY 13, 1950

THE SEPARATION AND IDENTIFICATION OF KETOACIDS BY FILTER PAPER CHROMATOGRAPHY

Sir:

In the course of studies on the amino acid metabolism of microörganisms carried out in this laboratory, it became desirable to isolate and identify minute quantities of ketoacids.

It was found that mixtures of ketoacids could be separated by unidimensional ascending partition chromatography on filter paper. The positions of the ketoacids on the chromatogram were made visible by spraying the paper (E. and D. 613), with an aqueous solution containing 0.1% semicarbazide hydrochloride and 0.15% sodium acetate, drying at 110°, and inspection under an ultraviolet lamp ("Mineralight").[1] The semicarbazones of the ketoacids appear as dark shadows on the faintly fluorescing paper. As little as 5 μg. of ketoacid could be demonstrated.

The approximate R_F values in a solvent system consisting of 95 parts n-butanol, 5 parts formic acid, saturated by shaking with water,

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[1] Chargaff, Magasanik, Doniger and Vischer, THIS JOURNAL, **71**, 1513 (1949).

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.

DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY
HARVARD MEDICAL SCHOOL BORIS MAGASANIK
BOSTON, MASS. H. EDWIN UMBARGER[5]

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).

[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.

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.

THE INSTITUTE OF PAPER CHEMISTRY
APPLETON, WISCONSIN IRWIN A. PEARL

RECEIVED FEBRUARY 23, 1950

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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).

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).