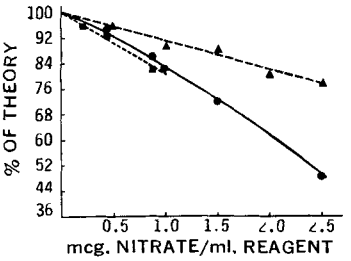


*Fig. 8—Effect of nitrate on reactions. Key: ▲, mestranol fluorometric assay; ●, mestranol colorimetric assay; ■, ethinyl estradiol colorimetric.*

centrations above 0.1 mcg./ml. of reagent as shown in Fig. 8.

## SUMMARY

The utility of $H_2SO_4$ to produce color or fluorescence of $\alpha$-ethinyl estradiol and derivatives has been established by several authors (1, 2, 5–7). When used in conjunction with a standardized reference tablet, the method yields optimum precision and accuracy for mestranol. The fluorescence measurement is more sensitive and more suitable for automation but less precise than the color measurement. Nitrate, nitrite, and hydrogen peroxide retard the reaction with sulfuric acid.

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*Keyphrases*

17$\alpha$-Ethynylestradiol-3-methyl ether (mestranol) tablets
Colorimetric analysis
Sulfuric acid–methanol color reagent
Automated fluorometric analysis
Fluorescence produced by sulfuric acid
Nitrate effect on analysis accuracy

# Notes

## Preparation of Some Phenyl Pyridyl Ethers with Antifungal and Antibacterial Properties

By RICHARD O. MUHLHAUSER* and EUGENE C. JORGENSEN†

**2-Methyl-4-chlorophenyl-4′-pyridyl ether (II) and 2-chlorophenyl-4′-pyridyl ether (III) were prepared by condensation of *N*-pyridyl-4-pyridinium chloride hydrochloride (I) with appropriate phenols. These compounds were found to be effective as antifungal agents but were less effective as antibacterial agents. Compound II had the greatest antifungal activity and the least toxicity.**

As PART of a program dealing with the synthesis of thyroxine analogs, a series of phenyl pyridyl ethers (II–VI) has been prepared. Two of these compounds, 2-methyl-4-chlorophenyl-4′-pyridyl ether (II) and 2-chlorophenyl-4′-pyridyl ether (III), were found to have antifungal and antibacterial activity.

The synthetic sequence utilized is shown in Scheme I.

*N*-Pyridyl-4-pyridinium chloride hydrochloride (I) was prepared as described by Jerchel (1). Compounds II–V were then prepared by condensing (I) with the appropriate phenols as described by Jerchel (2). Compound VI was prepared from V by acid hydrolysis using a mixture of concentrated hydrochloric and glacial acetic acids. Pertinent data are listed in Table I.

Nuclear magnetic resonance and infrared spectral studies supported the structures assigned to com-

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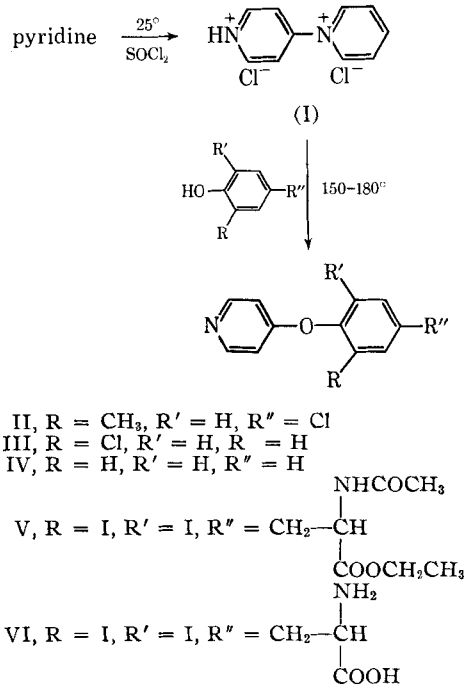
The authors thank Drs. Nancy C. Hall and Joseph Z. Krezanoski, Barnes-Hind Laboratories, Sunnyvale, Calif., for the assistance in obtaining the antifungal results shown in Table III.
This investigation was supported by grants AM 04223 and PHS 5 TI GM 0728 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
* Present address: School of Pharmacy, Oregon State University, Corvallis, OR 97331
† To whom correspondence should be addressed.




II, R = $CH_3$, R′ = H, R″ = Cl
III, R = Cl, R′ = H, R = H
IV, R = H, R′ = H, R″ = H

V, R = I, R′ = I, R″ = $CH_2$—CH(NHCOCH$_3$)—COOCH$_2$CH$_3$

VI, R = I, R′ = I, R″ = $CH_2$—CH($NH_2$)—COOH

*Scheme I*

pounds II-VI. In the case of compound V, the NMR spectrum was correlated with spectra obtained for substituted thyronines by Lehman and Jorgensen (3). A broad peak at approximately 8.5 p.p.m. was assigned to the $\alpha$ protons adjacent to the pyridyl nitrogen of V (4–5).

Antimicrobial and antifungal evaluation of compounds II and III was performed by the small-tube screening procedures of Catalfomo and Schultz (8) and by the paper disk diffusion method (9).

The data in Tables II and III illustrate the activity of these compounds against representative fungi and bacteria.

These results agree with those obtained by Hata *et al.* (10) and Tomita *et al.* (11), and suggest that both the pyridyl ring and the phenolic ether are operative in producing the antifungal activity.

Results from the screening tests showed that compound II completely inhibited the growth of *T. mentagrophytes* at a concentration of 0.142 mmoles/L. (31.2 mcg./ml.). Partial inhibition was obtained at a concentration of 0.045 mmoles/L. (10 mcg./ml.). Undecylenic acid at a concentration of 0.054 mmoles/L. (10 mcg./ml.) proved less effective.

Evaluation of antifungal activity by the paper disk method (9) showed that a concentration of 2 mg./ml. of II completely inhibited the growth of several other fungal species. Results obtained by this method are shown in Table III.

Compound II had no apparent effect on the mean survival time of mice infected with *Plasmodium berghei*, whereas compound III significantly decreased the life span of these mice. This indicates that compound II, the more effective antifungal agent, is the less toxic of the pair of compounds tested.

Compounds V and VI were synthesized for testing as possible thyromimetic compounds and were not tested for antifungal activity. They were found to be inactive as thyromimetics by the rat antigoiter assay method (12).

## EXPERIMENTAL

All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. The infrared spectra were determined with a Beckmann IR5 spectrophotometer by suspending the solid materials in potassium bromide disks. Nuclear magnetic resonance spectra were determined in deuterochloroform with tetramethylsilane as an internal standard. Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkely, Calif.

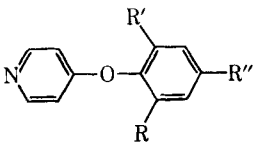
**Substituted Phenyl Pyridyl Ethers (Table I; II–IV) General Procedure**—A mixture of *N*-pyridyl-4-pyridinium chloride hydrochloride (1) (I, 15.0 Gm., 0.0645 mole) and the appropriate phenol (0.071 mole) was heated at 160–165° in an oil bath, forming a clear melt within a few minutes. On cooling, the residue was treated with 10% sodium carbonate solution and the dark-colored upper layer was removed and taken up in chloroform. The chloroform solution was extracted twice with 2 *N* sodium hydroxide solution (400 ml. total) and with water (800 ml.) and dried over sodium sulfate. After filtration, the chloroform was removed *in vacuo*, and the oil was purified by washing it several times with water (to remove pyridine) followed by distillation *in vacuo*. The clear distillates obtained crystallized after standing at room temperature for a few days. Pertinent data are listed in Table I.

**N-Acetyl-3,5-diiodo-4-(4′-pyridinoxy) Phenyl-L-alanine (V)**—Prepared from I (5 Gm., 0.0215 mole) and *N*-acetyl-3,5-diiodo-L-tyrosine ethyl ester (13) (11.92 Gm., 0.0237 mole). The mixture was fused at 165–180° as described above. The cooled, brittle, black solid was stirred with a hot solution of sodium carbonate (250 ml.) and the suspension was allowed to stand overnight. The next day, the polymeric-like suspension was treated again with hot sodium carbonate solution and insoluble material was collected by filtration. The gummy solid was dissolved in acetone, treated three times with decolorizing carbon,[1] and filtered. The solvent was concentrated and water added. A buff-white solid, m.p. 169-170°, was obtained. The product was recrystallized from acetone–water to give a white solid, m.p. 169-171°. A mixed melting point determination with *N*-acetyl-3,5-diiodo-L-tyrosine ethyl ester gave a depression in melting point. The solid was chromatographically homogeneous on Silica Gel H with an $R_f$ = 0.75 (ethyl acetate); $\bar{\nu}_{max.}$ in cm.$^{-1}$, 1257 (s), 1269 (s) (ether), 1669 (s) (amide), 1751 (vs) (ester), 3349 (m); NMR peaks at $\delta$ = 8.5 p.p.m. (broad) ($\alpha$ protons), $\delta$ = 7.65 p.p.m. (2,6 protons), $\delta$ = 6.7 p.p.m. ($\beta$ protons).

**3,5-Diiodo-4-(4′-pyridinoxy) Phenyl-L-alanine (VI)**—Prepared from compound V by acid hydrolysis. A mixture of V (0.736 Gm., 0.00127 mole), glacial acetic acid (24.5 ml.), and concentrated hydrochloric acid (16.36 ml.) was heated at reflux temperature for 3.5 hr. according to the method of Jorgensen and Slade (12). Additional

[1] Norit.

TABLE I—COMPOUNDS SYNTHESIZED



| Compd. | R | R′ | R″ | M.p., °C. | Formula | Anal., % Calcd. | Found |
|---|---|---|---|---|---|---|---|
| II | $CH_3$ | H | Cl | 53–55° | $C_{12}H_{10}ClNO$ | C, 65.61<br>H, 4.59<br>N, 6.38 | 65.42<br>4.51<br>6.56 |
| III[a] | Cl | H | H | 54–55 | $C_{11}H_8ClNO$ | | |
| IV[b,c] | H | H | H | 48 | $C_{11}H_9NO$ | | |
| V | I | I | $CH_2$—CH(NHCOCH$_3$)—COOCH$_2$CH$_3$ | 169–171 | $C_{18}H_{18}I_2N_2O_4$ | C, 37.26<br>H, 3.13<br>N, 4.83 | 37.33<br>3.33<br>4.80 |
| VI | I | I | $CH_2$—CH($NH_2$)—COOH | 216–217.5 | $C_{14}H_{12}I_2N_2O_3$ | C, 32.97<br>H, 2.37<br>I, 49.76 | 32.73<br>2.35<br>49.98 |

[a] Lit. (2) m.p. 54–55°. [b] Lit. (6) m.p. 44°. [c] Lit. (7) m.p. 48°.

TABLE II—ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF PHENYL PYRIDYL ETHERS

| Compd. | Concn., mmoles/L. | *T. mentagrophytes* 7 Day | *T. mentagrophytes* 14 Day | *Botrytis cinerea* 7 Day | *Botrytis cinerea* 14 Day | *Staphylococcus aureus* 48 hr. | *Escherichia coli* 48 hr. |
|---|---|---|---|---|---|---|---|
| II[a] | 1.14[b] | —[c] | — | — | — | — | — |
| | 0.57 | — | — | — | — | 4+ | 4+ |
| | 0.285 | — | — | 1+ | 2+ | 4+ | 4+ |
| | 0.228 | — | — | | | | |
| | 0.142 | +— | — | 3+ | 4+ | | |
| | 0.071 | 1+ | 2+ | | | | |
| | 0.045 | 1+ | 4+ | | | | |
| III[d] | 2.42 | — | — | — | — | — | — |
| | 1.21 | — | — | — | — | 4+ | 4+ |
| | 0.605 | — | — | 2+ | 4+ | | |
| | 0.302 | +— | +— | 3+ | 4+ | | |
| | 0.150 | 1+ | 2+ | 4+ | 4+ | | |
| Undecylenic acid | 0.54 | — | — | | | | |
| | 0.27 | — | — | | | | |
| | 0.054 | 3+ | 4+ | | | | |
| | 0.027 | 3+ | 4+ | | | | |
| | 0.00 (Alcohol control) | 3+ | 4+ | | | | |
| Griseofulvin (in dimethylformamide plus buffer)[e] | 0.142 | — | — | | | | |
| | 0.0284 | +— | 4+ | | | | |
| | 0.0142 | 1+ | 4+ | | | | |
| | 0.000 (DMF-buffer control) | 3+ | 4+ | | | | |

[a] 2-Methyl-4-chlorophenyl-4′-pyridyl ether. [b] Alcohol 5% or less plus organism. [c] +, growth; —, no growth; +—, uncertain growth. [d] 2-Chlorophenyl-4′-pyridyl ether. [e] Dimethylformamide plus buffer 5% or less, plus a DMF control plus sterility control.

hydrochloric acid (6 ml.) was added at the end of 1 hr. and again at the end of 2.5 hr. Additional glacial acetic acid (6 ml.) was added at the end of 2.5 hr. The cooled solution was filtered and the solvents removed *in vacuo*. The residue was dissolved in hot ethanol (50 ml.) and water (50 ml.) was added. After filtration, the pH was adjusted to 2.0; on cooling, crystals deposited. The crystals were redissolved by heating, and a hot 10% sodium acetate solution was added to bring the pH to 5.0. The solution was cooled and the precipitated white solid collected by filtration and dried; m.p. 229–231° dec. Two more isoelectric precipitations gave 0.38 Gm. (58% yield) of a white solid, m.p. 216–217.5° dec.; $\bar{\nu}_{max.}$ 820 (m), 860 (m), 1200 (ms), 1260 (m), 1400 (s), 1590 (s), 2800–3100 (b) (amino acid). Thin-layer chromatography on Silica Gel H using a solvent system of butanol–acetic acid–water

TABLE III—ANTIFUNGAL ACTIVITY OF 2-METHYL-4-CHLOROPHENYL-4′-PYRIDYL ETHER (II) AGAINST TEST ORGANISMS

| | Observations: 7 Days | Control | Ethyl Alcohol | 1/100 | 1/500 |
|---|---|---|---|---|---|
| 109 | *Epidermophyton floccosum* | ++[a] | ++ | ++ | ++ |
| 112 | *Microsporum audouinii* | ++ | +++ | 0 | 0 |
| 113 | *Microsporum canis* | ++ | ++ | 0 | 0 |
| 114 | *Microsporum gypseum* | +++ | ++++ | 0 | ++ |
| 116 | *Trichophyton gallinae* | ++++ | ++++ | + | ++++ |
| 119 | *Microsporum tonsurans* | ++ | ++ | 0 | 0 |
| 120 | *Trichophyton violaceum* | ++ | ++ | 0 | 0 |
| 121 | *Trichophyton mentagrophytes* | +++ | +++ | + | +++ |
| | 13 Days | | | | |
| 109 | *Epidermophyton floccosum* | ++ | +++ | +++ | ++ |
| 112 | *Microsporum audouinii* | ++ | +++ | 0 | 0? |
| 113 | *Microsporum canis* | +++ | +++ | 0 | +? |
| 114 | *Microsporum gypseum* | +++ | +++ | 0 | ++ |
| 116 | *Trichophyton gallinae* | ++++ | ++++ | + | ++++ |
| 119 | *Microsporum tonsurans* | +++ | +++ | 0 | 0 |
| 120 | *Trichophyton violaceum* | +++ | ++ | 0 | 0 |
| 121 | *Trichophyton mentagrophytes* | ++++ | ++++ | + | +++ |

[a] +, growth; 0, no growth; 0?, uncertain growth.

(100:30:10) showed a single spot (ninhydrin positive) for the white solid. The starting material (V) was negative to ninhydrin.

**Biological Materials and Methods**—The fungal species used were *Trichophyton mentagrophytes*, ATCC 9972, and *Botrytis cinerea*. The bacterial species employed were *Escherichia coli* and *Staphylococcus aureus* from the collections maintained at the department of Pharmacognosy, School of Pharmacy, University of California.[2]

The fungal organisms were maintained on Sabouraud dextrose medium[3] (liquid and agar), and the bacteria were maintained on nutrient medium[4] (broth and agar). The liquid cultures also served as a source of inocula.

For studies using *T. mentagrophytes*, the mycelial growth from 5–7-day old stationary cultures was homogenized in a sterile semimicro Waring blender for 30 sec. and added as 0.5 ml. inocula to the surface of an agar slant containing 9.5 ml. of Sabouraud's agar containing the test compounds. The tubes were incubated for 2 weeks at room temperature and growth was recorded at 7-day and 14-day intervals.

For antibacterial studies, actively growing 2–4-day old liquid cultures of *S. aureus* No. 209 P and *E. coli* No. 6522 were used as inocula. A bacterial suspension of 0.5 ml. was added to 9.5 ml. of nutrient broth No. 4A containing the dilutions of the compounds to be tested. The broth cultures were incubated for 48 hr. at 37°, and turbidity was then read to determine growth.

Results of the preliminary antifungal and antibacterial screening tests are shown in Table II.

In all cases, sterility controls were run for each sample of nutrient culture medium and for alcohol controls plus organism. Solutions of the agents to be tested were prepared in 95% ethanol and then diluted. In the case of griseofulvin, the compound was dissolved in dimethylformamide at various dilutions, and these dilutions were again diluted with phosphate buffer.

The second antifungal screening test on compound II was carried out in another laboratory.

Four sterile S and S absorbent disks (12.5 mm.) were placed in four Petri dishes containing Sabouraud's medium. The dishes were labeled as control, ethyl alcohol, $^1/_{100}$, and $^1/_{500}$. Compound II was dissolved in ethanol and dilutions of $^1/_{100}$ and $^1/_{500}$ were prepared. To the disks were added 2 drops of ethanol to one dish, 2 drops of $^1/_{100}$ dilution, 2 drops of $^1/_{500}$, and control was untouched. The disks were allowed to set 30 min. Then each set of disks was inoculated with a single culture of the respective organism and the plate was sealed with 3M Parafilm. The plates were then incubated at room temperature. Observations were taken at the end of 7 and 13 days. Results are shown in Table III.

**Antimalarial Test**[5]—Compounds II and III were administered subcutaneously in oil to mice at dose levels of 40, 160, and 640 mg./Kg. of body weight. The mice were infected with a lethal dose of *Plasmodium berghei* 3 days prior to administration of test compounds. With compound III, toxic deaths of all test animals were observed at all dose levels. No toxic deaths were observed with compound II, but the mean survival time of 6.2 days was not significantly prolonged.

[2] Obtained through the courtesy of Mrs. Evelyn Johnson and Dr. Robertson Pratt, Department of Pharmacognosy, School of Pharmacy, University of California Medical Center, San Francisco, Calif.

[3] Difco.

[4] Difco No. 4A.

[5] Antimalarial tests were performed at the Walter Reed Army Institute of Research, Washington, D. C.

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*Keyphrases*

Phenyl ethers—synthesis
Antibacterial activity of phenyl pyridyl ethers
Antifungal activity of phenyl pyridyl ethers
Antimalarial activity of phenyl pyridyl ethers
NMR spectrometry
IR spectrometry–structure
Microanalysis

# Analog Computer Simulation of Rheological Systems I Pseudoplastic Flow

By GERALD J. YAKATAN and OSCAR E. ARAUJO

**A method for the quantitative characterization of pseudoplastic systems using the analog computer was reported. Three parameters were obtained (one of which can be maintained constant for a given system) which completely describe the shape of rheograms for various CMC mucilages. The method developed can be carried out rapidly, the computer program is relatively simple, and the results should provide an easy means of communication among rheologists desiring to accurately compare experimental data on pseudoplastic systems.**

THE IMPORTANCE of pseudoplastic flow in pharmaceutical systems is apparent when one considers that most hydrocolloids and dilute suspensions exhibit this rheological pattern. A large number of pharmaceutical emulsions and semisolid preparations also behave as pseudoplastic systems.

The quantitative characterization of pseudoplasticity has been a difficult problem for the rheologist. The application of an empirical exponential equation involving the use of a log-log plot has been commonly accepted (1–3). However, the use of this equation has been questioned by several workers (4).

Other investigators have proposed various equations with a theoretical basis, but the involved calculations necessary to obtain the constant described considerably reduced their practical value (5–7).

In 1961 Shangraw *et al.* (8) proposed another equation in an attempt to provide a much better representation of non-Newtonian systems. The equation is:

$$F = f + \eta_\alpha S - b_\gamma e^{-aS} \qquad \text{(Eq. 1)}$$

where $F$ is the shearing stress, $S$ is the rate of shear, and $f$, $\eta_\alpha$, a, and $b_\gamma$ are constants characteristic of a particular system.

The problems involved in adequately characterizing pseudoplastic flow systems seemed to offer a challenging area of research. The concept of developing an equation with a minimum number of parameters which would completely characterize all pseudoplastic systems was an interesting one. It was also felt that the analog computer would be of great assistance in solving these problems.

A typical rheogram of a pseudoplastic substance appeared to be composed of a "first-order" and a "zero-order" segment. Consequently, a general equation combining the integrated expressions for a zero-order and a first-order process would be (9):

$$y = ax + b(1 - e^{-cx}) \qquad \text{(Eq. 2)}$$

where $y$ and $x$ are variables for the particular system and $a$, $b$, and $c$ are constants for the system.

For a specific rheological system, Eq. 2 becomes:

$$F = aS + b(1 - e^{-cS}) \qquad \text{(Eq. 3)}$$

where $F$ is the shearing stress, $S$ is the rate of shear, and $a$, $b$, and $c$ are constants. For purposes of computer programming, $F$ was made directly proportional to voltage and $S$ directly proportional to time, and Eq. 3 becomes:

$$V = at + b(1 - e^{-ct}) \qquad \text{(Eq. 4)}$$

## EXPERIMENTAL

The analog computer is rapidly becoming an extremely important tool in aiding the solution of complex scientific and engineering problems. In most modern computers, the continuous variables are d.c. voltages. The electronic analog computer makes possible the building of an electrical system in which d.c. voltages will vary with time in a manner similar to the variable of interest in the actual system under study.

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