synthesized as reported previously.[2] The preparation of MDP (C, 72.97; H, 10.19; N, 2.23) was the oxidation product of tripalmitoylpyridoxamine with nitrous acid.[2] Triacetylpyridoxamine was prepared as follows: Approximately 50 mg. of pyridoxamine dihydrochloride was refluxed in a mixture of glacial acetic acid (2.5 ml.) and acetic anhydride (2.5 ml.) for 1.5 hr. The solvent was then removed as much as possible *in vacuo*, and the residue was dissolved in a few ml. of absolute methanol. Upon addition of absolute ether containing dry hydrogen chloride, a precipitate was obtained. M.p. 129.0–130.0°.

*Anal.* Calcd. for $C_{14}H_{18}N_2O_5 \cdot HCl$: N, 8.47. Found: N, 8.67.

Other triacylpyridoxamines were prepared from pyridoxamine dihydrochloride and the respective acid chlorides in a manner similar to the one described for the synthesis of the tripalmitoyl derivative. *N,O,O*-Tridecanoylpyridoxamine was recrystallized from absolute methanol. M.p. 86.5–87.5°.

*Anal.* Calcd. for $C_{38}H_{66}N_2O_5$: C, 72.33; H, 10.54; N, 4.44. Found: C, 72.62; H, 10.33; N, 4.54.

*N,O,O*-Tribenzoylpyridoxamine was recrystallized from 60% ethanol. M.p. 131.0–133.0°.

*Anal.* Calcd. for $C_{29}H_{24}N_2O_5$: N, 5.83. Found: N, 5.98.

*N,O,O*-Tri-*p*-nitrobenzoyl pyridoxamine was recrystallized from pyridine-methanol. M.p. 202.0–203.0°.

*Anal.* Calcd. for $C_{29}H_{21}N_5O_{11}$: N, 11.38. Found: N, 11.34.

DEPARTMENT OF FOOD TECHNOLOGY
UNIVERSITY OF ILLINOIS
URBANA, ILL.

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# *p*-Phenylazobenzoyl Chloride for Identification and Chromatographic Separation of Colorless Compounds. III. Phenols

E. O. WOOLFOLK AND J. M. TAYLOR[1]

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The value of *p*-phenylazobenzoyl chloride as a reagent for identification and chromatographic separation of alcohols and amines has been shown in previous communications.[2,3] Since the reactions of alcohols, amines, and phenols are similar in many respects, it seemed desirable to study the usefulness of *p*-phenylazobenzoyl chloride as a reagent for phenols.

Whereas alcohols and amines reacted readily with the reagent to give good yields of esters and amides respectively, in general, the phenols reacted with difficulty and gave small yields of esters. The procedure used in the preparation of the derivatives was very simple. It consisted of heating for 4 hours a solution of the phenol and acid chloride in pyridine. Longer periods of reaction time did not increase the yield of derivatives. With the exception of the derivatives of 2-methylphenol, 3,4-dimethylphenol, 2,4-dimethylphenol, 2,6-dimethylphenol, and 3-ethyl-5-methylphenol all were precipitated from the reaction mixture as solids. Although the yields were low they were adequate for easy identification. The *p*-phenylazobenzoates are highly crystalline derivatives which are easily purified. The melting points of the derivatives are high enough so that only the derivative of 2,6-dimethylphenol was obtained as an oil, and they are separated widely enough to ensure identification. In those several instances where the melting points of derivatives were similar, mixture melting points showed considerable depression and wide spreads.

The aryl-*p*-phenylazobenzoates that have been characterized are recorded in Table I.

The derivatives of phenols which are commonly used for identification are neither colored nor fluorescent. Hence they are not suitable to applications of chromatographic adsorption for the separation of phenol derivatives. The brilliantly colored aryl-*p*-phenylazobenzoates have been found to be suitable derivatives for separation of mixtures by chromatographic adsorption techniques. The adsorbents used in preparing preliminary columns for the separation of the derivatives were as follows: alumina, alumina-celite mixture, silicic acid, and silicic acid–celite. Of these adsorbents silicic acid–celite was the most satisfactory. It afforded better separations of mixtures of derivatives, faster percolation rate and easier desorption of the derivatives. In the case of alumina various percentages of alcohol in Skellysolve B or benzene were required to develop the chromatograms, and to desorb the derivatives it was necessary to heat the adsorbent with a solution of 80% alcohol-water. Under these conditions of desorption the derivatives underwent hydrolysis and transesterification to give a mixture from which the aluminum salt of *p*-phenylazobenzoic acid and ethyl-*p*-phenylazobenzoate were identified.

Table II shows the results obtained by the adsorption of 15 pairs of aryl *p*-phenylazobenzoates on mixtures of alumina-celite and silicic acid–celite. The first member of each separable pair listed in Table II was the most strongly adsorbed derivative. Six pairs marked +++ were separated sufficiently on at least one of the adsorbents to make two zones visible with a colorless zone between. Two pairs marked ++ formed a continuous band on at least one of the adsorbents. Sectioning of this with subsequent elution yielded an almost homogeneous top and bottom section with intervening sections of varying composition. Three pairs marked + formed a continuous band on at least one of the adsorbents, but sectioning of this gave impure top and bottom materials which were of different melting points, showing that a mixture was initially present. Finally, eight pairs marked "minus" gave no separation on one or both of the adsorbents as there was no significant difference in the melting points of the material from the top and bottom sections of the continuous band. Those pairs which

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[1] This paper is based on work presented by J. M. T. in partial fulfillment of requirements for an undergraduate research course offered in the Department of Chemistry of Central State College.

[2] E. O. Woolfolk, F. E. Beach, and S. P. McPherson, *J. Org. Chem.*, **20**, 391 (1955).

[3] E. O. Woolfolk and E. H. Roberts, *J. Org. Chem.*, **21**, 436 (1956).

TABLE I

ESTERS OF *para*-PHENYLAZOBENZOIC ACID

| Phenol Used | M.P., °C.[a] Corrected | Yield,[b] % | Formula | Nitrogen[c] Calcd. | Nitrogen[c] Found |
|---|---|---|---|---|---|
| Phenol | 148.0–150.0 | 45* | $C_{19}H_{14}N_2O_2$ | 9.27 | 9.30 |
| 2-Methylphenol | 110.0–111.5 | 17 | $C_{20}H_{16}N_2O_2$ | 8.86 | 8.68 |
| 4-Methylphenol | 134.5–136.5 | 13 | $C_{20}H_{16}N_2O_2$ | 8.86 | 8.70 |
| 2,3-Dimethylphenol | 134.0–136.0 | 14 | $C_{21}H_{18}N_2O_2$ | 8.48 | 8.33 |
| 3,4-Dimethylphenol | 104.0–107.0 | 26 | $C_{21}H_{18}N_2O_2$ | 8.48 | 8.23 |
| 2,4-Dimethylphenol | 110.0–113.0 | 20 | $C_{21}H_{18}N_2O_2$ | 8.48 | 8.61 |
| 2,5-Dimethylphenol | 95.5– 97.5 | 24 | $C_{21}H_{18}N_2O_2$ | 8.48 | 8.30 |
| 3,5-Dimethylphenol | 104.5–106.5 | 36 | $C_{21}H_{18}N_2O_2$ | 8.48 | 8.53 |
| 2,6-Dimethylphenol | Oil | | | | |
| 4-Ethylphenol | 117.0–118.0 | 48 | $C_{21}H_{18}N_2O_2$ | 8.48 | 8.57 |
| 3-Ethyl-5-methylphenol | 159.0–161.0 | 9 | $C_{22}H_{20}N_2O_2$ | 8.14 | 8.31 |
| 2-Isopropyl-5-methylphenol (thymol) | 85.0– 88.0 | 9 | $C_{23}H_{22}N_2O_2$ | 7.82 | 7.82 |
| *o*-Chlorophenol | 120.0–121.0 | 55 | $C_{19}H_{13}ClN_2O_2$ | 8.32 | 7.99 |
| *m*-Chlorophenol | 127.5–128.5 | 36 | $C_{19}H_{13}ClN_2O_2$ | 8.32 | 8.15 |
| *p*-Chlorophenol | 153.0–154.0 | 13 | $C_{19}H_{13}ClN_2O_2$ | 8.32 | 8.34 |
| *o*-Bromophenol | 126.5–127.5 | 80 | $C_{19}H_{13}BrN_2O_2$ | 7.35 | 7.15 |
| *m*-Bromophenol | 124.5–126.0 | 58 | $C_{19}H_{13}BrN_2O_2$ | 7.35 | 7.11 |
| *p*-Bromophenol | 167.5–168.5 | 32 | $C_{19}H_{13}BrN_2O_2$ | 7.35 | 7.22 |
| 2,4,6-Tribromophenol | 116.0–119.0 | 8 | $C_{19}H_{11}Br_3N_2O_2$ | 5.20 | 5.09 |
| 2,4,6-Tribromo-3-methylphenol | 130.0–132.0 | 34* | $C_{20}H_{13}Br_3N_2O_2$ | 5.07 | 4.98 |
| *o*-Iodophenol | 125.5–127.5 | 8 | $C_{19}H_{13}IN_2O_2$ | 6.54 | 6.65 |
| *o*-Nitrophenol | 136.5–137.0 | 30 | $C_{19}H_{13}N_3O_4$ | 12.10 | 10.39 |
| *m*-Nitrophenol | 160.5–162.5 | 33 | $C_{19}H_{13}N_3O_4$ | 12.10 | 10.79 |
| *p*-Nitrophenol | 203.0–206.0 | 9 | $C_{19}H_{13}N_3O_4$ | 12.10 | 10.15 |
| *p*-Phenylphenol | 213.5–214.0 | 50* | $C_{25}H_{18}N_2O_2$ | 7.40 | 7.44 |
| *o*-Phenylphenol | 141.0–144.0 | 5 | $C_{25}H_{18}N_2O_2$ | 7.40 | 7.34 |
| *p*-Phenylazophenol | 213.5–214.0 | 19 | $C_{25}H_{18}N_4O_2$ | 13.79 | 13.89 |
| α-Naphthol | 118.0–119.0 | 20 | $C_{23}H_{16}N_2O_2$ | 7.95 | 8.09 |
| β-Naphthol | 190.0–193.0 | 18 | $C_{23}H_{16}N_2O_2$ | 7.95 | 7.82 |
| 2-Carbomethoxyphenol (methyl salicylate) | 100.5–102.5 | 17 | $C_{21}H_{16}N_2O_4$ | 7.77 | 7.76 |

[a] Melting points taken on Kofler Micro Hot Stage. [b] Yields marked with asterisk are on products purified only by recrystallization; otherwise reported on products purified by chromatography then recrystallized. [c] Microanalyses by the Dumas method were performed by the Du Good Chemical Laboratories, St. Louis, Mo.

were not investigated on one of the adsorbents are marked O in the appropriate column.

TABLE II

CHROMATOGRAPHIC SEPARATION OF ESTERS OF *p*-PHENYLAZOBENZOIC ACID

| Mixture[a] | | Alumina-Celite | Silicic Acid–Celite |
|---|---|---|---|
| *o*-Bromophenol | *o*-Iodophenol | 0 | +++ |
| *o*-Bromophenol | *m*-Bromophenol* | – | – |
| *o*-Bromophenol | *p*-Bromophenol | – | – |
| *m*-Bromophenol | *p*-Bromophenol | – | – |
| *m*-Chlorophenol | *m*-Bromophenol* | – | +++ |
| *o*-Chlorophenol | *o*-Bromophenol* | – | +++ |
| *o*-Chlorophenol | *o*-Iodophenol | 0 | +++ |
| *m*-Chlorophenol | *p*-Chlorophenol | 0 | – |
| *o*-Chlorophenol | *p*-Chlorophenol | +++ | +++ |
| *m*-Chlorophenol | *o*-Chlorophenol | ++ | +++ |
| *p*-Methylphenol | *o*-Methylphenol | ++ | ++ |
| α-Naphthol | β-Naphthol | – | – |
| *o*-Nitrophenol | *m*-Nitrophenol* | – | + |
| *p*-Nitrophenol | *o*-Nitrophenol | 0 | + |
| *o*-Phenylphenol | *p*-Phenylphenol | 0 | + |

[a] The pairs marked with an asterisk on desorption of the bands from alumina-celite gave materials which did not melt below 300° (aluminum salt of *p*-phenylazobenzoic acid).

Previous chromatographic studies[3,4] have shown that adsorption affinities of *ortho*-substituted benzenes are markedly less than those observed for the *meta* and *para* isomers and that the *meta* and *para* isomers have about the same adsorption affinities. However, it was found that within an *ortho*, *meta*, and *para* series of the derivatives prepared in this work no appreciable differences were exhibited in their relative adsorption affinities (see Table II). Further, in the case of three pairs (*o*-nitrophenol, *m*-nitrophenol; *o*-chlorophenol, *p*-chlorophenol; *o*-phenylphenol, *p*-phenylphenol) it was found that the *ortho* isomer was the most strongly adsorbed derivative. An examination of these experiments indicates the order of influence of a halogen substituent on the degree of adsorption affinity of these derivatives to be Cl > Br > 1.

## EXPERIMENTAL

*Reagents.* 2-Methylphenol, 4-methylphenol, 2,3-dimethylphenol, 2,4-dimethylphenol, 2,5-dimethylphenol, 3,5-di-

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(4) For a recent study of steric factors as a definite influence in the behavior of *ortho*-substituted benzenes on chromatographic columns see J. K. Carlton and W. C. Bradbury, *J. Am. Chem. Soc.*, **78**, 1069 (1956).

methylphenol, 4-ethylphenol, and 3-ethyl-5-methylphenol were kindly furnished by Dr. R. A. Friedel, U. S. Bureau of Mines, Bruceton, Pa.

2,4,6-Tribromophenol and 2,4,6-tribromo-3-methylphenol were prepared as described by Shriner and Fuson.[5]

The remaining phenols were commercially available grades and were used without further purification, except phenol and 1-naphthol which were redistilled.

The solvents Skellysolve B and benzene were redistilled, and ethyl alcohol and ethyl acetate were used as purchased.

The adsorbents used in preparing the chromatographic columns were silicic acid (Mallinckrodt, prepared by the method of Ramsey and Patterson), alumina (Aluminum Company of America, Grade F-20) and celite-535 (Johns-Manville). *p*-Phenylazobenzoyl chloride is manufactured by Distillation Product Industries.

*Preparation of aryl p-phenylazobenzoates.* A mixture of acid chloride (approximately 0.1 g.), phenol ($0.0003M$ excess), and 3 to 6 ml. of pyridine was refluxed gently for 4 hr. The reaction product was poured with stirring into ice and 50 ml. of 10% sodium carbonate solution. If the crude reaction product separated as a solid, it was filtered off and washed with water and dried. The crude product was dissolved in Skellysolve B or a mixture of Skellysolve B and benzene and chromatographed on a mixture of silicic acid–celite (2 to 1 by weight) on which the free acid was strongly adsorbed. The derivative was then recrystallized. Chromatography of the crude derivatives of the following phenols gave two colored bands with a colorless band in between: 2,3-dimethylphenol, 3,4-dimethylphenol, *o*-chlorophenol, *m*-chlorophenol, *p*-chlorophenol, *o*-bromophenol, *m*-bromophenol, *p*-bromophenol, *o*-nitrophenol, *m*-nitrophenol, and *p*-nitrophenol. Of these two bands the lower one gave sharp melting points and good analyses. When the reaction product separated out as an oil, it was extracted with ether. The ether extract was washed successively with water and a saturated sodium chloride solution, dried over sodium sulfate, and the ether was removed. The residue was chromatographed, then recrystallized. The red-colored esters crystallized from Skellysolve B or mixtures of Skellysolve B and benzene as crystalline solids or fine needles.

*Chromatographic separations.* A typical chromatographic separation of a mixture of two components was conducted as described below. A tube 20 mm. × 400 mm. was connected to a suction flask. A 50 to 50 mixture by volume of alumina and celite or a 2 to 1 mixture by weight of silicic acid and celite was prepared for use as the adsorbent. While tapping the sides of the tube with cork rings, the tube was filled with the adsorbent to a height of approximately 290 mm. Then full suction of the water aspirator was applied to the suction flask which caused the adsorption column to decrease to approximately 268 mm. in height. The adsorbent was then wetted with Skellysolve B. In order to obtain a suitable percolate rate it was necessary to apply full suction with silicic acid and celite but only partial suction was required for alumina and celite.

The mixture of esters (10 to 20 mg. of component) was dissolved in the minimum volume of warm Skellysolve B or solutions of benzene in Skellysolve B and was adsorbed on the column. The chromatogram was developed by passing Skellysolve B, then solutions of benzene in Skellysolve B, and finally solutions of ethyl acetate in Skellysolve B through the adsorbent. The adsorbent was dug out of the column by a long narrow spatula and eluted with absolute ethanol if the adsorbent was silicic acid–celite or 95% ethanol if it was alumina-celite. When a continuous band was obtained, the band was arbitrarily dug out in several sections. The pure components were obtained from the top and bottom sections whereas the intervening sections were mixtures. The eluents were concentrated, filtered into a tared flask, and the last traces of solvent removed *in vacuo* under a stream of nitrogen. Melting points of the residues were determined.

*Acknowledgment.* We are indebted to the National Science Foundation for financial aid in the pursuance of this work.

CHEMISTRY DEPARTMENT
CENTRAL STATE COLLEGE
WILBERFORCE, OHIO

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[5] R. C. Shriner and R. C. Fuson, *Identification of Organic Compounds.* John Wiley and Sons, Inc., New York, N. Y., 2nd ed., 1947, p. 174.

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## Reduction with Hydroxylating the Organism, *Curvularia lunata*

DAVID GOULD, JAN ILAVSKY, RITA GUTEKUNST, AND
E. B. HERSHBERG

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With the aim of obtaining a 14α-hydroxy derivative, 4,6-pregnadiene-17α,21-diol-3,11,20-trione[1] was incubated with *C. lunata,* an organism reported to hydroxylate certain steroids at C-7,[2] C-11[3] and C-14.[4] Paper chromatography showed two major spots absorbing ultraviolet light, of which only the less polar stained with triphenyltetrazolium reagent.[5] The more polar substance thus no longer had the dihydroxy acetone side chain.

Column chromatography permitted isolation of both compounds. The less polar was shown by infrared spectrum and melting point to be the starting material. The more polar substance, obtained in 20–25% yield was considered to be a 17,20,21-triol on the basis of the strong polarity and the altered staining chracteristics, since such reductions have been observed with other microorganisms.[6] The substance analyzed for 4,6-pregnadiene-17α,20ξ,21-triol-3,11-dione with methanol of crystallization, and had infrared and ultraviolet spectra and a molecular rotation change which agreed with the assigned structure.

Acetylation gave a diacetate confirming introduction of a new acylable hydroxyl group. The direction of the molecular rotation change, +556, supports the structure and demonstrates[6] that the transformation product is 4,6-pregnadiene-17α,-20β,21-triol-3,11-dione. The evidence thus confirms this conversion as a reduction by an organism

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