was obtained, a yield of about 90% based on the amount of formaldehyde and hydrochloric acid used. The crude THPC melted at about 145°. The pure compound, which melts at 151°, can be obtained by recrystallizing from acetic acid.[3]

At about 25°, the absorption of phosphine and its reaction with formaldehyde and hydrochloric acid are rapid until about 85 to 90% of these materials are converted to THPC. In order to avoid the escape of unused phosphine, which is likely to occur near the end of the reaction, the reaction should be discontinued when an amount of THPC has been made that is equivalent to about 85 to 90% of the formaldehyde originally present in the solution. This end-point can be determined conveniently by removing a small sample of the solution, evaporating the volatile components and weighing the crystalline THPC. The maximum temperature at which the evaporation can be carried out and still obtain crystals is not known, but when the solution is evaporated on a hot-plate at relatively high temperatures, a viscous sirup is obtained which is difficult, if not impossible, to crystallize. The sirup is probably a mixture of THPC and tris-(hydroxymethyl)-phosphine oxide since it is known that phosphonium compounds are converted to phosphine oxides by heat.

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(4) One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

Some Halophenoxyacetic Anhydrides

BY GEORGE SVARNAS AND WILLIAM L. HOWARD

RECEIVED AUGUST 30, 1954

Interest in the herbicidal properties of 2,4-dichlorophenoxyacetic acid and related compounds[1] suggested that the preparation of the anhydrides of some phenoxyacetic acids might prove useful. Accordingly, three representative anhydrides have been prepared: 2,4-dichlorophenoxyacetic anhydride, 4-fluorophenoxyacetic anhydride and 2,4-difluorophenoxyacetic anhydride. Thompson, *et al.*,[2] have reported 2,4-dichlorophenoxyacetic anhydride, but, since no details are given, its preparation is presented herewith. 4-Fluorophenoxyacetyl chloride and 2,4-difluorophenoxyacetyl chloride, prepared as intermediates, and the corresponding amides have not been reported previously.

Qualitative observations during the preparation of these compounds indicated that the acid chlorides and anhydrides are intermediate in reactivity between the corresponding derivatives of the typical aromatic and aliphatic acids. Because of their sensitivity to hydrolysis, moisture was carefully excluded throughout the preparations.

It is of interest to note that phenoxyacetic acid melts at a considerably higher temperature than its anhydride. The anhydrides reported here exhibit the same relationship.

All pertinent data concerning the compounds prepared are listed in Table I. The melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected.

Experimental

Preparation of the Acid Chlorides.—The acid[3] is refluxed with about two equivalents of thionyl chloride for 2–3 hours. After the excess thionyl chloride has been removed at the aspirator the residual liquid is vacuum distilled.

Preparation of the Amides.—A benzene solution of the acid chloride is added portionwise with stirring to a flask containing benzene into which dry ammonia is being passed, an excess of ammonia being maintained during the entire addition. After being allowed to stand overnight the mixture is filtered and the solid is washed thoroughly with water. Concentration of the benzene filtrate yields more product, after which the crops are combined and recrystallized from benzene or acetone–benzene.

Preparation of the Anhydrides.—The silver salt of the phenoxyacetic acid is prepared by converting the acid to the sodium salt with the equivalent amount of aqueous sodium hydroxide, after which the equivalent amount of aqueous silver nitrate is added to the hot solution with vigorous stirring. The precipitated silver salt is filtered off without delay, washed successively with water, ethanol, and ether, and then dried *in vacuo*.

Slightly more than one mole of the freshly prepared, finely divided silver salt is refluxed with a mole of the acid chloride in ligroin (d. 0.72–0.74) for one to three days. The hot supernatant liquid then is decanted into a flask, the flask is stoppered, and the solution is allowed to cool to room temperature during which time an oil separates and eventually solidifies. The solid product is then recrystallized from benzene–ligroin.

TABLE I

Compound	M.p. or b.p. (mm.), °C.	Carbon, % Calcd.	Carbon, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Yield, %
4-$FC_6H_4OCH_2COCl$	106–107 (9)	50.94	50.80	....	....	3.21	3.06	73.3
2,4-$F_2C_6H_3OCH_2COCl$	96 (7)	46.51	46.51	....	....	2.44	2.57	69.0
4-$FC_6H_4OCH_2CONH_2$	109.5–110.5	...	...	8.29	8.35	..	..	75.5
2,4-$F_2C_6H_3OCH_2CONH_2$	123.5–125	...	...	7.49	7.55	..	..	83.0
$(4\text{-}FC_6H_4OCH_2CO)_2O$	54.5–56	59.63	59.86	161[a]	159[a]	3.75	3.93	47.5
$(2,4\text{-}F_2C_6H_3OCH_2CO)_2O$	73.5–74.5	53.66	53.69	179[a]	177[a]	2.81	2.97	75.0
$(2,4\text{-}Cl_2C_6H_3OCH_2CO)_2O$	76.5–77.5	45.32	45.52	212[a]	212[a]	2.38	2.31	42.5

[a] Saponification equivalent.

(1) M. E. Synerholm and P. W. Zimmerman, *Contrib. Boyce Thompson Inst.*, **14**, 91 (1945); R.L. Weintraub, *J. Agr. Food Chem.*, **1**, 250 (1953).

(2) H. E. Thompson, C. P. Swanson and A. G. Norman, *Botan. Gaz.*, **107**, 476 (1946).

(3) The fluorophenoxyacetic acids were prepared from the corresponding phenols obtained from the Illinois State Geological Survey. The 2,4-dichlorophenoxyacetic acid was obtained from the Eastman Kodak Co.

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Rates of Aminolysis

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Table I records first-order rate constants for the solvolysis of seven compounds in various amine solutions. The results are striking from the point of view of the Brönsted catalysis law for bases. The ratio of rate constants for *n*-butylamine and aniline

(1) National Science Foundation Fellow, 1952–1953. For further experimental data see D. C. Dittmer, Ph.D. thesis, M.I.T., September, 1953.

is 13.0 for methyl bromide, 2.3 for *n*-butyl bromide, 0.89 for benzyl chloride, 0.0008 for benzhydryl chloride and 10^{-5} for trityl thiocyanate. Thus the higher electrophilic reactivity of aniline outweighs its lower basicity and nucleophilic reactivity with the benzyl, benzhydryl and trityl compounds.

TABLE I

RATES OF AMINOLYSIS

Compound[a]	Solvent[b]	Temp., °C.	k_1, sec.$^{-1}$
MeBr	Et_3N	50	1.56×10^{-4}
MeBr	*n*-$BuNH_2$	50	1.00×10^{-1}[c]
MeBr	C_5H_5N	50	8.30×10^{-3}
MeBr	$PhNH_2$	50	7.66×10^{-3}
MeBr	$PhNMe_2$	50	2.69×10^{-4}
n-BuBr	Et_3N	75	2.81×10^{-7}
n-BuBr	*n*-$BuNH_2$	75	2.03×10^{-3}
n-BuBr	C_5H_5N	75	5.13×10^{-4}
n-BuBr	$PhNH_2$	75	8.79×10^{-4}
n-BuBr	PhNHMe	75	2.57×10^{-4}
n-BuBr	$PhNMe_2$	75	3.79×10^{-6}
n-BuBr	*m*-$ClPhNH_2$	75	9.24×10^{-5}
i-BuBr	*n*-$BuNH_2$	50	2.92×10^{-5}
i-BuBr	C_5H_5N	50	3.15×10^{-6}
i-BuBr	$PhNH_2$	50	8.26×10^{-6}
$PhCH_2Cl$	Et_3N	50	7.55×10^{-8}
$PhCH_2Cl$	*n*-$BuNH_2$	50	1.12×10^{-3}
$PhCH_2Cl$	C_5H_5N	50	1.28×10^{-4}
$PhCH_2Cl$	$PhNH_2$	50	1.26×10^{-3}
$PhCH_2Cl$	PhNHMe	50	5.04×10^{-4}
$PhCH_2Cl$	$PhNMe_2$	50	1.73×10^{-7}
$(Ph)_2CHCl$	Et_3N	25	$<10^{-10}$[d]
$(Ph)_2CHCl$	*n*-$BuNH_2$	25	9.91×10^{-7}
$(Ph)_2CHCl$	C_5H_5N	25	1.93×10^{-7}
$(Ph)_2CHCl$	$PhNH_2$	25	1.20×10^{-3}
$(Ph)_2CHCl$	PhNHMe	25	2.31×10^{-4}[e]
$(Ph)_2CHCl$	*m*-$ClPhNH_2$	25	1.03×10^{-2}[f]
$(Ph)_3CSCN$	*n*-$BuNH_2$	25	$<10^{-8}$[g]
$(Ph)_3CSCN$	C_5H_5N	25	$<10^{-10}$[h]
$(Ph)_3CSCN$	$PhNH_2$	25	2.2×10^{-3}

[a] Ph = C_6H_5 or *p*-substituted C_6H_4. [b] Me, Et, Bu = CH_3, C_2H_5, C_4H_9; C_5H_5N = pyridine. The solvent was always 95.2% amine–4.8% benzene based on volumes before mixing. [c] Estimated from points beyond 90% reaction. [d] 1.3% reaction after 2.4×10^5 min. (*ca.* 8 months). [e] Value is approximate because of analytical difficulties. (Oxidation of product to colored material obscured end points.) [f] Accurate to only about 20%. The reaction was difficult to follow because of its speed. [g] No appreciable reaction after 9.1×10^4 min. (2 months). [h] No appreciable reaction after 3.3×10^5 min. (7.5 months).

Table II lists some half-lives for the reaction of *m*-chloroaniline with benzhydryl chloride in benzene solution. The rate of reaction seems to be proportional to a higher than first power of the concentration of *m*-chloroaniline. Addition of small amounts of *n*-butylamine accelerated the reaction in spite of the fact that *n*-butylamine has a lower dielectric constant (5.3) than *m*-chloroaniline (13.3). This acceleration may be due to *n*-butylamine acting as a nucleophilic reagent while the *m*-chloroaniline behaves as an electrophilic reagent in a concerted "push-pull" process.

TABLE II

REACTION OF *m*-CHLOROANILINE AND BENZHYDRYL CHLORIDE IN BENZENE AT 75°

m-$ClPhNH_2$, *M*	$(Ph)_2CHCl$, *M*	*n*-$BuNH_2$, *M*	Temp., °C.	$t_{1/2}$, min.
1.061	0.092	...	75	420
0.237	.083	...	75	25,000
.257	.121	...	75	19,000
.252	.119	0.040	75	3,750

Experimental

Solvents.—*n*-Butylamine from Carbide and Carbon Chemical Co. was dried with calcium hydride and distilled through a 5-foot column, packed with glass helices, b.p. 78–79°, $n^{25}D$ 1.3950. The amine was stored in a glass-stoppered bottle over calcium hydride. Triethylamine from Sharples Chemicals, Inc., was distilled from potassium hydroxide flakes through the 5-foot column, b.p. 91°. It was stored in a glass-stoppered bottle over calcium hydride.

Mallinckrodt reagent grade pyridine was distilled from sodium hydroxide through the 5-foot column, b.p. 114–115°. Eastman White Label *m*-chloroaniline was distilled, b.p. 228–229°, $n^{23}D$ 1.5932. Mallinckrodt reagent grade aniline was distilled from zinc dust through the 5-foot column, b.p. 184°. All were stored in tightly capped brown bottles over calcium hydride.

Other Materials.—Methyl bromide from the Westvaco Co., 99.5% pure, was used without further purification. *n*-Butyl bromide, Eastman Kodak Co. White Label grade, was dried over calcium hydride and distilled, b.p. 90.2–91°, $n^{25}D$ 1.4330. Benzyl chloride was Merck reagent grade used without further purification.

Benzhydryl chloride was prepared according to Gilman and Kirby from benzhydrol and thionyl chloride.[2] Benzhydrol was prepared from benzophenone and zinc dust.[3]

Kinetic Measurements.—In most of the aminolysis studies 40 ml. of amine was allowed to come to the thermostat temperature, and 2 ml. of a stock solution of the halide in dry benzene was added. Aliquots were taken as required. In slow reactions the reaction mixture was pipetted into soft glass test-tubes which had been drawn out to give a neck for easy sealing. The tubes were sealed and placed in a constant temperature bath, one tube being opened at once and analyzed to give a zero-time point. In the reactions of methyl bromide with pyridine and triethylamine, smaller amounts of amine and halide were used; and, because of the speed of the reaction, a reaction was done for each point, the entire reaction mixture being quenched and the ionic halogen determined.

The reaction solutions were quenched in separatory funnels containing cold, 50% nitric acid and benzene. The benzene layer was extracted at least twice with water, and the aqueous solution of bromide or chloride was titrated by the Volhard method. In the determination of chloride, nitrobenzene was added after precipitation of silver chloride to coagulate the precipitate. All reaction vessels and pipets were dried before use.

Calculation of Rate Constants.—The rate constant, k_1, for a first-order reaction is $-(2.303/t) \log (1 - z)$ where t is the time and z is the fraction reacted. Values of k_1 for the aminolyses were obtained by plotting $10 (1 - z)$ on a log scale *vs.* t on an arithmetic scale from which $k_1 = 0.693/$ half-life. If the line plotted did not go exactly through the origin, a line parallel to it which did was used, and the half-life was determined from the new line.

Determination of Products.—There is ample evidence in the literature that, at the temperatures used in this study, the primary halides isobutyl and *n*-butyl bromide, do not yield olefins.[4-6]

The kinetics indicated that the reaction of halides with primary amines gave only secondary amines, which was to be expected considering the large excess of amine over halide.

The product from the reaction of aniline and benzhydryl chloride was N-benzhydrylaniline, which separated from ether as a resin, m.p. 47–50°. It was identified as its nitrate and hydrochloride, m.p. 155–156° and 201–203°, respec-

(2) H. Gilman and J. Kirby, THIS JOURNAL, **48**, 1733 (1926).

(3) F. Y. Wiselogle and H. Sonneborn, III, "Organic Syntheses," Coll. Vol. 1, Ed. 2, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 90.

(4) J. Semb and S. M. McElvain, THIS JOURNAL, **53**, 690 (1931).

(5) W. Drake and S. M. McElvain, *ibid.*, **56**, 1810 (1934).

(6) C. Noller and R. Dinsmore, *ibid.*, **54**, 1025 (1932).

tively.[7] On treatment with nitrous acid it gave a yellow-green oil which showed a positive Liebermann nitroso test.[8] The product gave no test for a primary amine after diazotization and treatment with β-naphthol.

The reaction of benzhydryl chloride with *n*-butylamine gave an amine which yielded a crystalline hydrochloride, m.p. 261–263°.

Anal. Calcd. for $C_{17}H_{22}NCl$ (N-benzhydryl-*n*-butylamine hydrochloride): C, 74.02; H, 8.04; N, 5.08; Cl, 12.86. Found: C, 73.92; H, 8.02; N, 5.70; Cl, 12.28.

(7) H. Gilman, J. E. Kirby and C. R. Kinney, THIS JOURNAL, **51**, 2206 (1929); A. Skita, *Ber.*, **48**, 1696 (1915); W. E. Bachmann, THIS JOURNAL, **53**, 2674 (1931); M. Busch, *Ber.*, **37**, 2693 (1904).

(8) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," Ed. 3, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 114.

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Hydroxylation of Desoxycorticosterone with *Neurospora crassa*[1]

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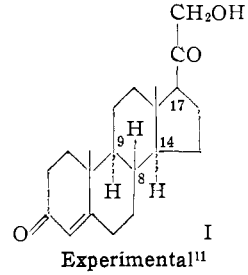
Since the original report[4] on the microbiological hydroxylation of progesterone to 11α-hydroxyprogesterone by means of *Rhizopus arrhizus*, a variety of microörganisms and substrates have been investigated.[5] In connection with studies under way in these laboratories to investigate the metabolism of steroids by *Neurospora crassa*, we have examined the action of this mold on a variety of steroids. It should be noted that with the exception of only a brief general statement[6] no reports have appeared concerning the steroid transforming capacity of this mold.

Pilot experiments with *Neurospora crassa*, indicated by paper chromatographic analysis that most of the steroids tested were transformed to a large extent into more polar products. These findings indicated that *Neurospora crassa* has versatile enzymatic capacities to effect modifications of the steroid molecule; the nature of the precise alterations produced awaits isolation and identification of the products obtained. In the case of desoxycorticosterone (I) it was possible to isolate one of the transformation products in crystalline form; the present note is concerned with a description of these experiments.

Incubation of 3 g. of I in 18 l. of medium by the procedure outlined in the experimental section, led to several crystalline fractions. The most abundant one was isolated in sufficient quantity (*ca.* 10–20%) so that its chemical constitution could be defined to a considerable extent. This product (m.p. 182–184°, $[\alpha]_D$ + 163°) possessed the empirical formula $C_{21}H_{30}O_4$, thus indicating that an additional hydroxyl group had been introduced. The presence of the unaltered Δ^4-3-keto moiety and the ketol side chain was demonstrated by the ultraviolet and infrared spectral data and the typical red color produced with triphenyltetrazolium chloride.[7] Oxidation with sodium bismuthate[8] led to a crystalline etio acid, $C_{20}H_{28}O_4$, which represented additional chemical proof for the ketol side chain.

The infrared spectrum (kindly determined by Mr. Paul Skogstrom, Worcester Foundation), differed from those of the following monohydroxylated derivatives of desoxycorticosterone; 2α, 6 α, 6 β, 11 α, 11 β, 14 α, 15 α, 16 α, 17 α and 19. The mobility of the hydroxylated desoxycorticosterone approximated that of corticosterone and a further similarity between the two substances was demonstrated by the fact that just as with corticosterone, the new product yielded only a monoacetate. This acetate still exhibited a free hydroxyl band in the infrared and possessed a mobility on paper which was essentially identical with that of corticosterone 21-acetate. In conjunction with our previous studies, the nature of the newly introduced hydroxyl group was indicated by its resistance toward chromium trioxide, which demonstrated that only three tertiary positions (8, 9 or 14 β) were open for the location of the hydroxyl group. If microbiological hydroxylations in the steroid series do not proceed *via* an intermediate double bond (as appears to be the case in the 11 β hydroxylation in adrenal tissue[9]) it is reasonable to assume tentatively (first suggested to us by Dr. B. M. Bloom) that a hydroxyl group enters only those spatial positions in the steroid nucleus where a hydrogen atom was present originally. On this basis positions 9 β, 8 α and 14 β are unlikely, leaving either the 8 β- or 9 α-positions as the location for the hydroxyl group in the presently described x-hydroxydesoxycorticosterone.[10]



I

Experimental[11]

Incubation of Desoxycorticosterone with *Neurospora crassa*.—A wild strain of *Neurospora crassa* (No. 74A) was

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(2) The Worcester Foundation for Experimental Biology.

(3) Wayne University.

(4) D. H. Peterson and H. C. Murray, THIS JOURNAL, **74**, 1871 (1952).

(5) *Cf.* K. Florey, *Chimia*, **8**, 81 (1954); O. Hanc and E. Riedl-Tumova, *Pharmazie*, **9**, 877 (1954); D. H. Peterson in "Perspectives and Horizons in Microbiology," edited by S. A. Waksman, Rutgers U. Press, New Brunswick, N. J., 1955, p. 121.

(6) H. C. Murray and D. H. Peterson, U. S. 2,602,769, p. 62.

(7) *Cf.* A. Zaffaroni, *Recent Progr. Hormone Research*, **8**, 51 (1953).

(8) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(9) M. Hayano and R. I. Dorfman, *J. Biol. Chem.*, **211**, 227 (1954).

(10) After the isolation of our hydroxylated derivative of desoxycorticosterone, Dr. D. H. Peterson of the Upjohn Co., kindly supplied us with a number of monohydroxylated derivatives of desoxycorticosterone for comparison purposes. One of these, tentatively designated as 8β-hydroxydesoxycorticosterone, isolated by Dr. P. D. Meister (Upjohn Co.) as a bioconversion product of 11-hydroxydesoxycorticosterone by *Mucor parasiticus*, has been found to have an identical infrared spectrum, m.p. 182–184°, and specific rotation $[\alpha]_D$ +167°, (*C*, 0.709 in chloroform) with the corresponding physical constants found for our compound. Clear-cut assignment of the hydroxyl group to position 8 or 9, has not yet been made.

(11) Melting points were determined on the Kofler block and rotations were measured in chloroform solution. The microanalyses were carried out by Geller Laboratories, Hackensack, New Jersey.