alone or admixed with material prepared by the other route.

Anal. Calcd. for $C_{15}H_{14}N_2OS$: N, 10.36. Found: N, 10.08.

N-(*m*-Tolyl)-N'-phenylthiourea.—Prepared by mixing and allowing to spontaneously react *m*-tolyl isothiocyanate and aniline, or *m*-toluidine and phenyl isothiocyanate; m. p., after three recrystallizations from benzene–Skellysolve B, 109–110°, alone or when admixed. Otterbacher and Whitmore[3] allowed *m*-toluidine and phenyl isothiocyanate to react in alcohol and obtained a product of the correct nitrogen analysis but with m. p. 94°.

Anal. Calcd. for $C_{14}H_{14}N_2S$: C, 69.39; H, 5.82; N, 11.56; S, 13.23. Found: C, 69.53; H, 5.86; N, 11.34; S, 13.47.

(3) Otterbacher and Whitmore, THIS JOURNAL, **51**, 1909 (1929).

WARNER INSTITUTE FOR THERAPEUTIC RESEARCH
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RECEIVED APRIL 24, 1950

Substituted Salicylaldehydes and Derivatives[1]

For the study of some special physical properties of chelated metal salts of certain Schiff bases of aldehydes a number of substituted salicylaldehydes and their derivatives were prepared, purified and analyzed. The results are presented in Table I.

TABLE I

Salicylaldehyde	Method[9]	Yield, %	M. p., °C.[6]	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
3-Methyl[2]	A	6	En, 115	73.0	72.9	6.75	6.63
4-Methoxy,[3] methyl ether	D	>50	DNPH, 245	52.0	52.2	4.05	4.10
5-Methoxy[4]	A	16	DNPH, 211–212	59.8	60.0	4.98	5.02
3-Chloro[5]	C	40	En, 150–152	57.0	57.0	4.15	4.27
5-Chloro[7]	[10]	<5	En, 174–175	57.0	57.1	4.15	4.22
3-Iodo	B	5[8]	Cu salt	30.1	30.9	1.44	1.57
3-Cyano	[11]	5–10	114	65.3	65.2	3.4	3.4
3-Formyl, oxime[7]	A	<5	NPH, 269–271	56.0	56.6	4.0	4.0
3-Phenyl	A	13	50	78.7	78.3	5.05	5.16
5-*t*-Butyl	A	~10	En, 165–167.5	75.8	76.1	8.42	8.34
3-Isopropyl-6-methyl[2]	A	16	En, 112–113	75.8	75.3	8.42	8.38
6-Isopropyl-3-methyl[2]	A	20	En, 139–140	75.8	75.4	8.42	8.08

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA LLOYD N. FERGUSON[12]
BERKELEY, CALIF. MELVIN CALVIN

RECEIVED OCTOBER 3, 1949

(1) The work reported here was done under contract OEM sr/276 between the National Defense Research Committee and the University of California during the period April, 1942, to April, 1944. For the primary interest of the project, see papers I through VII, THIS JOURNAL, **69**, 1886 (1947).

(2) J. C. Duff, *J. Chem. Soc.*, 547 (1941).

(3) L. Gattermann, *Ber.*, **31**, 1149 (1898).

(4) F. Tiemann and W. H. M. Muller, *ibid.*, **14**, 1990 (1881).

(5) H. H. Hodgson and T. A. Jenklnson, *J. Chem. Soc.*, 1740 (1927).

(6) En = ethylenediamine Schiff base; DNPH = 2,4-dinitrophenylhydrazone; NPH = *p*-nitrophenylhydrazone.

(7) H. Voswinckel, *Ber.*, **15**, 2023 (1892).

(8) There was also obtained a 13% yield of 3-iodo-4-hydroxybenzaldehyde.

(9) A = Duff; B = Reimer–Tiemann, *Ber.*, **9**, 824 (1876); C = Kolbe–Schmitt and NaHg reduction, THIS JOURNAL, **68**, 2502 (1946); D = Ferguson, *Chem. Revs.*, **38**, 230 (1946).

(10) Chlorination with sodium hypochlorite.

(11) From 3-methylsalicylaldehyde to the oxime, m. p. 97–99°, acetylation and bromination to 2-acetoxy-3-cyanobenzal bromide, m. p. 98–99°, and hydrolysis with sodium carbonate solution. DNPH, m. p. 270° (dec.)

(12) Chemistry Dept., Howard University, Wash., D. C.

o-Nitrophenyl-β-D-galactopyranoside and its Tetraacetate

These derivatives of D-galactose were prepared for use as chromogenic substrates for studies on bacterial β-galactosidases.[1]

***o*-Nitrophenyl-β-D-galactopyranoside Tetraacetate.**—The procedure of Glaser and Wulwek[2] for the corresponding glucose derivative was employed. Forty-two grams of *o*-nitrophenol was dissolved in a solution of 16.8 g. of sodium hydroxide in 420 ml. of water. To this was added a solution of 88 g. of tetraacetyl-α-D-galactopyranosyl bromide[3] in 620 ml. of acetone. After standing at room temperature for five hours the solvent was removed by distillation under reduced pressure. The product appeared as long needles which caused considerable bumping. It was filtered off and the concentration continued until no more crystals formed. After recrystallization from 95% ethanol 56 g. was obtained, m. p. 172–172.5°, $[\alpha]^{18}D$ + 69.9° (*c*, 1.9, chloroform).

Anal. Calcd. for $C_{20}H_{23}O_{12}N$: C, 51.1; H, 4.90. Found: C, 51.0; H, 4.96.

***o*-Nitrophenyl-β-D-galactopyranoside.**—The free glycoside was obtained by catalytic deacetylation. One gram of the above product was suspended in 50 ml. of absolute methanol and 1 ml. of 0.4 *N* barium methoxide solution was added. The mixture was refrigerated and shaken periodically. After four hours a clear solution resulted and soon thereafter crystals in the form of long hair-like needles separated. After twenty-four hours the reaction mixture was concentrated under reduced pressure and a quantitative yield of *o*-nitrophenyl-β-D-galactopyranoside was obtained. The melting point after two recrystallizations from absolute ethanol was 193–194°, $[\alpha]^{18}D$ −51.9° (*c*, 1.0, water).

Anal. Calcd. for $C_{12}H_{15}O_8N$: C, 47.8; H, 4.98. Found: C, 48.1; H, 5.20.

(1) By Dr. J. Lederberg, Department of Genetics, University of Wisconsin.

(2) Glaser and Wulwek, *Biochem. Z.*, **145**, 514 (1934); see also Babers and Goebel, *J. Biol. Chem.*, **105**, 473 (1934); Aizawa, *Enzymologia*, **6**, 321 (1939).

(3) Haynes and Todd, *J. Chem. Soc.*, 303 (1950).

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WISCONSIN MARTIN SEIDMAN
MADISON, WISCONSIN KARL PAUL LINK

RECEIVED MAY 25, 1950

5-Chloro-2-pyrimidinethiol

5-Chloro-2-pyrimidinethiol.—A solution of 13 g. (0.33 mole) of sodium hydroxide in 400 cc. of methanol was saturated with hydrogen sulfide. Fifty grams (0.33 mole) of 2,5-dichloropyrimidine[1] was added and the mixture was refluxed for fifteen minutes. Violent bumping followed

(1) English, Clark, Shepherd, Marson, Krapcho and Roblin, THIS JOURNAL, **68**, 1039 (1946).

the rapid separation of sodium chloride. The salt was filtered and the filtrate was diluted with 800 cc. of water. The resulting partially cloudy mixture was acidified with hydrochloric acid and the yellow precipitate of 5-chloro-2-pyrimidinethiol collected and dried: 37.5 g. (76%), m. p. 218–223°. After crystallization from methanol the melting point was constant at 221–222°.

Anal.[2] Calcd. for $C_3H_2ClN_2S$: C, 32.8; H, 2.0; S, 21.8. Found: C, 33.0, 33.0; H, 2.3, 2.4; S, 22.1, 22.0.

The yellow color of the compound could not be removed by treatment in alkaline solution with hydrosulfite, zinc dust, charcoal, or a combination of the last two agents.

(2) Carried out under the direction of Dr. J. A. Kuck.

CHEMOTHERAPY DIVISION
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AMERICAN CYANAMID COMPANY JACKSON P. ENGLISH
STAMFORD, CONNECTICUT ESTHER B. LEFFLER

RECEIVED MARCH 31, 1950

COMMUNICATIONS TO THE EDITOR

BOUNDARY SPREADING IN SEDIMENTATION VELOCITY EXPERIMENTS

Sir:

The only case in which sedimentation velocity experiments have been successfully used for size distribution analysis is the one in which no significant amount of diffusion occurs during the course of the experiment.[1] This situation does not ordinarily obtain, and in this Communication we present in outline a method to sort out the contributions of heterogeneity and diffusion to the spreading of the sedimentation boundary, thus providing means for the computation of each.

Since the second moments about the mean are additive in a combined distribution composed of independent distributions[2] we may derive the expression

$$\sigma^2 = \sigma_0^2 + 2Dt + \bar{x}^2\left[p\omega^2t + \frac{(p\omega^2t)^3}{3!} + \frac{(p\omega^2t)^5}{5!} + \ldots\right]^2 \quad (1)$$

Thus, and to a good approximation, we have

$$\frac{\sigma^2 - \sigma_0^2}{2t} = D + \frac{p^2\omega^4}{2}\bar{x}^2t$$

In these equations, σ^2 and σ_0^2 are the second moments of the curve which defines the sedimenting boundary at times $t = t$ and $t = 0$, D is the weight-average diffusion constant, ω is the angular velocity of the ultracentrifuge rotor, p is the standard deviation of the sedimentation constant distribution and $\bar{x}$ may be taken as the distance from the center of rotation to the centroidal ordinate of the boundary. This equation shows that when the apparent diffusion coefficient $(\sigma^2 - \sigma_0^2)/2t$, is plotted against $\bar{x}^2t$, a straight line is obtained with intercept D and slope $p^2\omega^4/2$. A drift of "diffusion coefficient" with time has long been recognized as a test of homogeneity with respect to sedimentation behavior.[3]

(1) *Cf.* for example, W. B. Bridgman, THIS JOURNAL, **64**, 2349 (1942).

(2) C. E. Weatherburn, "A First Course in Mathematical Statistics," Cambridge University Press, Cambridge, 1946, p. 82.

(3) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940, p. 287.

The distribution function, $g(s)$ which gives the relative amount of the molecular species with s_{20} of s is given by

$$g(s) = \frac{dn}{dx}\left(\frac{x}{x_0}\right)^2\frac{x\omega^2t}{n_1 - n_2}\frac{\eta_{20}}{\eta_t} \quad (2)$$

when diffusion is negligible.[4] When diffusion is not negligible, an "apparent distribution" defined in this manner may be extrapolated to infinite time to give the actual distribution of sedimentation constants, since the spreading of the boundary due to differences in s is proportional to $\bar{x}t$, while the spreading due to diffusion is proportional to $t^{1/2}$, as is shown by equation (1).

The method has been applied in the analysis of sedimentation velocity diagrams for pepsin-digested γ-globulin systems from horse anti-diphtheric serum. Additional information has been obtained, not only as regards the actual size distribution in these systems, but also with respect to the mechanism of the enzymatic degradation. A definitive account of these studies will be submitted at a later date.

Grateful acknowledgment is made to the U. S. Public Health Service and to the Wisconsin Alumni Research Foundation.

(4) R. Signer and H. Gross, *Helv. Chim. Acta*, **17**, 726 (1934).

DEPARTMENT OF CHEMISTRY
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MADISON, WISCONSIN J. W. WILLIAMS

RECEIVED AUGUST 7, 1950

SYNTHESIS AND ISOLATION OF A CRYSTALLINE SUBSTANCE WITH THE PROPERTIES OF A NEW B VITAMIN

Sir:

A naturally occurring factor active for *Leuconostoc citrovorum* 8081 has been described,[1,2] and a synthetic reaction mixture derived from folic acid has been reported to be active for this organism.[3]

We wish to report the synthesis and isolation

(1) Sauberlich and Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

(2) Broquist, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **71**, 549 (1949).

(3) Shive, *et al.*, THIS JOURNAL, **72**, 2818 (1950).