index must be at least partially due to peroxide formation.

As a result of the above observations, all propionaldehyde samples[2] were distilled and collected in a nitrogen atmosphere, and the Pyrex receivers were boiled out with water for twenty to thirty minutes and thoroughly dried in an oven. The boiling procedure may remove an adsorbed oxygen layer from the glass surface.

Carefully cleaned copper, zinc, aluminum, tin, and stainless steel surfaces showed reaction when immersed in the aldehyde. The evidence of reaction, surface corrosion, coloration of solution, and crystalline solids in the solution was less pronounced in the oxygen-free aldehyde samples than in those which gave positive peroxide tests. It has been assumed that these reactions were due to the formation of an acid.

During preliminary determinations of the density of propionaldehyde at −64.2°, it was observed that while the pycnometer was warming to room temperature, a relatively large volume of gas was evolved. A moderate quantity of a white solid, which melted near −20°, appeared to be the source of the gas bubbles. The pycnometer was closed with ground glass caps after filling, so continued absorption of oxygen from the atmosphere could not have occurred. When the pycnometer had been thoroughly flushed with dry nitrogen before it was filled with aldehyde, the solid and gas bubbles were not observed. The peroxide would be more stable at the lower temperatures and when warmed would decompose with liberation of oxygen.

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A Condensation Reaction of Thiols with Phenol Alcohols

BY I. W. RUDERMAN AND E. M. FETTES

We wish to report that thiols condense directly with phenol alcohols when treated with hydrogen chloride or boron trifluoride in the absence of water. This previously unrecorded reaction, which may be represented by the formula

$$o\text{- or } p\text{-(OH)RCH}_2\text{OH} + \text{R'SH} \longrightarrow \text{(OH)RCH}_2\text{SR'} + \text{H}_2\text{O}$$

where R is an aryl and R′ is an aryl or alkyl group, offers a convenient new synthesis of pure methylene thioethers of phenols in high yield. The preparation of such thioethers was recently reported[1] to be possible either through the reaction of a phenol, formaldehyde and a thiol (from which reaction it should be difficult to isolate a pure product because of isomer and polymer formation), or through the condensation of an *o*- or *p*-(dialkylaminomethyl)-phenol[2] with a thiol.

The synthesis of 2-hydroxy-α^1,α^3-bis-(butylmercapto)-mesitylene was accomplished as follows. One-half mole of 1-butanethiol and 0.05 mole of 2-hydroxy-α^1,α^3-mesitylenediol were dissolved in 125 ml. of glacial acetic acid, and dry hydrogen chloride gas was passed into the solution for two hours. The temperature rose seventeen degrees during the first fifteen minutes of the reaction, and then dropped gradually to room temperature. The reaction vessel was stoppered and set aside for two days. Water was added, and the oil which separated out was washed with water and then steam-distilled to remove unreacted thiol. A yellow oil remained, and it was distilled in a molecular still at a pressure of less than 1 micron. Ten and four-tenths grams (60% yield) of a clear, pale yellow liquid, $n^{20}\text{D}$ 1.5490, was collected when the pot temperature was 100-105°.

Anal. Calcd. for $C_{17}H_{28}OS_2$: C, 65.33; H, 9.03; S, 20.52. Found: C, 65.40; H, 9.00; S, 20.46.

The phenyl urethan derivative was prepared according to the method of Shriner and Fuson[3]; colorless crystals, m. p. 92.8-93.2°, were obtained after one recrystallization from petroleum ether–chloroform.

Anal. Calcd. for $C_{24}H_{33}NO_2S_2$: C, 66.78; H, 7.71; N, 3.25; S, 14.86. Found: C, 67.04; H, 7.67; N, 3.34; S, 15.43.

By analogy with the condensation of phenols with phenol alcohols under similar conditions,[4] the condensation of phenol di- or trialcohols with polythiols should yield polymers.

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[1] R. F. McCleary and S. M. Roberts, U. S. Patent 2,322,376, June 22, 1943. No preparation of a *pure* thioether is given.

[2] H. A. Bruson and C. W. MacMullen, THIS JOURNAL, **63**, 270 (1941).

[3] R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 2nd edition, John Wiley and Sons, Inc., New York, N. Y., 1940, p. 136.

[4] J. B. Niederl and I. W. Ruderman, THIS JOURNAL, **67**, 1176 (1945).

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A Counter-Current Distribution System for the Separation and Determination of Streptomycin Types

BY G. W. E. PLAUT[1] AND R. B. MCCORMACK

As a means of extraction and purification of streptomycin, it was found that a 5% stearic acid solution in Pentasol[2] would extract streptomycin from an aqueous concentrate at *p*H 9. However, when a double extraction was made with this system, the distribution coefficient of this second extraction was appreciably lower than the first (Table I). The streptomycin content was determined by conversion to maltol.[3]

These observations indicated mixtures of streptomycin types in our original solution. A method of separation of these types into pure preparations or for analysis using counter-current procedures was suggested by these data.

For analytical purposes, applying the Craig counter-current technique,[4] the distribution co-

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[1] Present address: Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wisconsin.

[2] A mixture of synthetic amyl alcohols made by Sharples Chemicals, Inc.

[3] J. R. Schenck and M. A. Spielman, THIS JOURNAL, **67**, 2276 (1945).

[4] L. C. Craig, *J. Biol. Chem.*, **155**, 519 (1944).

TABLE I

SOLVENT EXTRACTION OF STREPTOMYCIN CONCENTRATE

Sample	Extraction no.	$K = \frac{C_{\text{Solvent}}}{C_{\text{Spent Aqueous}}}$
A	1	28.3
	2	19.3
B	1	31.0
	2	17.1
C	1	27.6
	2	10.2

efficients must be modified to approach the range $K = 0.3$ to 8 so that suitable distribution curves can be obtained employing a 24-plate distribution. This was accomplished by sodium bicarbonate addition to reach *p*H 7.6. A solvent system for the Craig technique using butanol and 5% *p*-toluenesulfonic acid has been described[5] but separation of types with this system is not sufficient to give other than a broad distribution curve. With the system here described, the separation of streptomycin from mannosidostreptomycin is sufficient to produce two distinct curves suitable for simple calculation (Fig. 1) with no evidence of tautomerism as described using the *p*-toluenesulfonic acid–butanol system.[6] The peak tube for mannosidostreptomycin is usually at tube 9–11 with streptomycin having a peak ten plates beyond indicating a distribution coefficient for streptomycin about seven times greater than mannosidostreptomycin with this system and concentration.

Procedure.—The immiscible liquid pair consists of the aqueous phase containing 0.5% sodium bicarbonate C. P. and 1.0% sodium chloride of C. P. and a solvent phase (Pentasol) containing 5% stearic acid U. S. P. (Baker and Adamson). These two solutions are prepared fresh daily and mutually saturated in a separatory funnel prior to using in the apparatus.

The streptomycin sample is dissolved in a portion of the aqueous system to approximate 1 mg. (free base)/ml. and 8 ml. of this used to fill the bottom part of tube O. All other tubes of the bottom part of the machine are filled with the prepared aqueous phase (8 ml. per tube). After the top of the instrument has been fitted, 8 ml. of the solvent phase is added to each tube. The procedure of operation is then followed as usual for the Craig machine, a mixing time of two minutes and a separation time of about seven to ten minutes being allowed, depending upon the appearance of the guide tube or examination of the system in the machine. After the distribution has been completed, the tubes are completely emptied into small separatory funnels using a siphon–vacuum arrangement; 0.2 ml. of 7 N sulfuric acid and 8 ml. of benzene are added to each and the funnels vigorously agitated for ten minutes. This procedure ensures the displacement of the streptomycin from the solvent phase into the aqueous phase which is then separated from the solvent mixture and the concentration of streptomycin in each tube is determined. While bio-assays can be employed after suitable neutralization and dilution, the more suitable procedure for detection of small proportions of either types of antibiotic has been by the maltol procedure using the ultraviolet absorption measurement at 325 mμ following the method previously described.[6]

Calculations.—Recoveries of total streptomycins are calculated from the summation of ΔD values of each tube.

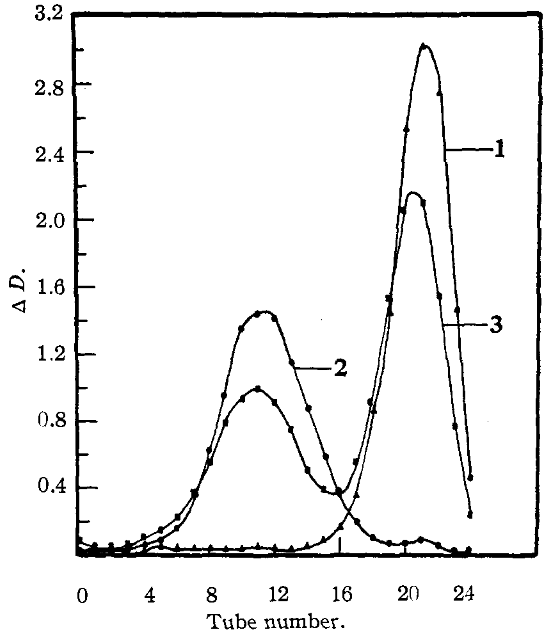

Fig. 1.—Counter-current distribution of streptomycin types: 1, streptomycin; 2, mannosidostreptomycin with 2.8% streptomycin; 3, mixed preparation containing 55% streptomycin and 45% mannosidostreptomycin.

These are usually between 80 and 90%, in agreement with the ΔD of the introduced sample. If mannosidostreptomycin is present, its content is estimated by the summation of ΔD values of this area of the distribution (from minimal point to minimal point). To determine the percentage weight content in relation to the total sample correction for the molecular weight differences of the two streptomycins is made by multiplying the total ΔD values of mannosidostreptomycin by 1.12.

The utility of the above described procedure has been fully shown not only for documentation of commercial production batches of streptomycin but also for laboratory studies on the isolation and characterization of streptomycin types.[6,7,8]

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[5] E. Titus and J. Fried, *J. Biol. Chem.*, **168**, 393 (1947).

[6] E. Titus and J. Fried, *ibid.*, **174**, 57 (1948).

[7] J. Fried and E. Titus, THIS JOURNAL, **70**, 3615 (1948).

[8] L. J. Heuser, M. A. Dolliver and E. T. Stiller, *ibid.*, **70**, 2833 (1948).

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The Physical Properties of Five Isomeric Methyl-*t*-butylcyclohexanes

BY K. T. SERIJAN, P. H. WISE AND L. C. GIBBONS

The syntheses and physical properties of *o*-, *m*- and *p*-*t*-butyltoluenes have been recently reported from this Laboratory.[1] In this communication, we wish to present the results obtained from the catalytic hydrogenation of these hydrocarbons including data on the separation of the geometric isomers and the physical properties and analyses of the five methyl-*t*-butylcyclohexanes which were

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[1] Serijan, Hipsher and Gibbons, THIS JOURNAL, **71**, 873 (1949).