TABLE I

SOLVENT EXTRACTION OF STREPTOMYCIN CONCENTRATE

Sample	Extraction no.	$K = \frac{C_{Solvent}}{C_{Spent\ Aqueous}}$
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.

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

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

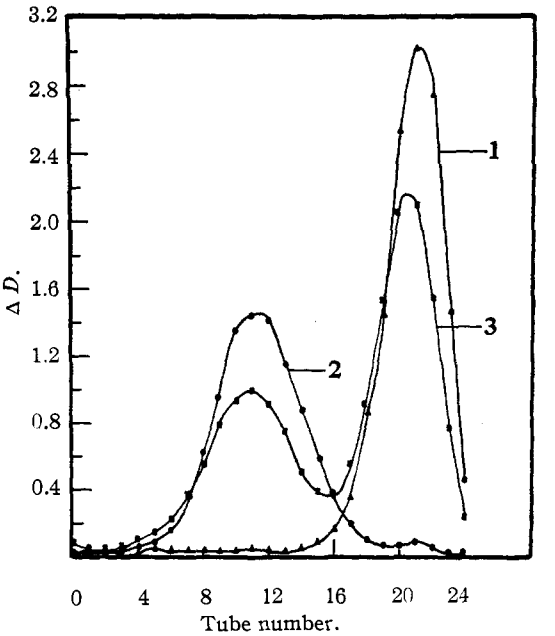

Fig. 1.—Counter-current distribution of streptomycin types: 1, streptomycin; 2, mannosidostreptomycin with 2.8% streptomycin; 3, mixed preparation containing 55% streptomycin and 45% mannosidostreptomycin.

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]

(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

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

TABLE I

PHYSICAL PROPERTIES, YIELDS AND ANALYSES OF FIVE ISOMERIC METHYL-*t*-BUTYLCYCLOHEXANES

Isomer	B. p., °C. (760 mm.)	M. p., °C.	d^{20}, g./ml.	$n^{20}D$	Yield,[d] %	Heat of combustion,[e] kcal./mole	Analyses, % C[f]	H[g]
1,2-[a]	193.67	−60.20	0.83151	1.4565	100	1609	85.85	14.15
1,3-[b]	184.09	−46.11	.80829	1.4460	75	1602	85.74	14.36
1,3-[c]	187.58	−66.02	.81757	1.4486	25	1602	85.80	14.40
1,4-[b]	186.72	−51.04	.80252	1.4418	58	1597	85.27	14.31
1,4-[c]	188.74	−35.03	.81718	1.4487	42	1603	85.85	14.29

[a] Obtained in relatively low purity as indicated by melting curve. [b] Low boiling isomer. [c] High boiling isomer. [d] Relative yield of each isomer not including small amounts of aromatic hydrocarbons found in end cuts. [e] Experimental net values obtained by use of a Parr oxygen bomb calorimeter and following the procedure in ASTM Designation D240-39. [f] Calculated 85.63%. [g] Calculated 14.37%.

obtained. None of these compounds has been previously described in the literature.

In connection with the 1,2-compound, it was of special interest to observe the effect of a highly branched group on the formation of the *cis* and *trans* isomers. Theoretically, due to steric hindrance, it is quite probable that only one isomer may be obtained exclusively or at least in predominant yield. As shown in Table I, apparently only one isomer was obtained since the product had a constant refractive index value throughout the entire distillation range. No definite conclusions can be reached, however, since a high degree of purity could not be obtained despite several attempts at purification described below. In this particular case, the difficulty may readily be attributed to the proximity of boiling points coupled with the similarity of structure in the possible isomers. The melting-point method was used in the evaluation of purity and the remaining two pairs of geometric isomers are reported in rather high purity (above 97 mole per cent.).

Since the appropriate thermodynamic properties have not been investigated, no specific assignment can be made at this time with reference to the definite identification of the *cis* and *trans* configurations. It has been shown[2,3] that the general application of von Auwers' rule[4] is susceptible to error as indicated by the recent change in the name of the *cis* and *trans* isomers of 1,3-dimethylcyclohexane. Accordingly, the products isolated by fractionation are referred to in Table I as the low and high boiling isomers. The physical properties were determined by methods previously described[5] with the exception of the density values which were measured using the apparatus and procedure described by Forziati, *et al.*[6]

Experimental[7]

Materials and Procedure.—The aromatic hydrocarbons used in this investigation had physical properties identical with those previously reported.[1] The hydrogenation charges were 425 g. (2.87 moles), 688 g. (4.64 moles) and 931 g. (6.28 moles) for the *o*-, *m*- and *p*- compounds, respectively. The volume of methylcyclohexane used as solvent was equal to that of the hydrocarbon in each case and 20% by weight of U.O.P. nickel was used as catalyst. Hydrogenation occurred at 170–190° at approximately 1500 p.s.i. and was essentially quantitative.

Purification.—The separation of the isomers was effected by fractionation using a seven-foot Podbielniak column rated above 100 theoretical plates. Subsequent purification, where necessary, consisted of azeotropic distillation with Methyl Carbitol and all the products were passed through silica gel columns prior to the determination of physical properties.

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(2) Pitzer and Beckett, *ibid.*, **69**, 978 (1947).
(3) Rossini and Pitzer, *Science*, **105**, 647 (1947).
(4) von Auwers, *Ann.*, **420**, 92 (1920).
(5) Gibbons, *et al.*, THIS JOURNAL, **68**, 1130 (1946).
(6) Forziati, Mair and Rossini, *J. Research Natl. Bur. Standards*, **35**, 513 (1945).
(7) Macro analyses and heats of combustion by Mr. A. M. Busch and physical properties by Mr. J. F. Thompson of this Laboratory.

Light Absorption of Aqueous Hydrogen Peroxide Solutions in the Near Ultraviolet Region[1]

BY ROBERT C. TAYLOR AND PAUL C. CROSS

It is well established that the ultraviolet absorption spectrum of hydrogen peroxide in aqueous solution consists of a continuum with no evidences of structure extending from about 3800 Å. out to beyond 2000 Å. However, there has been some disagreement as to the exact magnitude of the absorption coefficients, and data in the range between 3200 and 3800 Å. are rather meager. Moreover, the most concentrated solution for which data are available is only about 35% by weight. In connection with other work, the ultraviolet absorption of 50 and 91% by weight solutions of hydrogen peroxide in water has been measured in the region from 2700 to 3800 Å. and the data are reported here.

Experimental.—The source of hydrogen peroxide was 90% material of high purity donated by the Buffalo Electrochemical Company. Before use it was made 0.003 normal in sodium hydroxide and distilled *in vacuo* under its own vapor pressure. By maintaining the liquid at 30° and cooling the receiver in dry ice–isopropyl alcohol, one may distill the solution without ebullition and thus preclude the carry-over of dust and spray particles. The solutions were diluted when necessary with redistilled dust-free water. Analysis of the solutions was carried

(1) This work was supported in part by the Office of Naval Research under Contract N6ori-88, Task Order No. 1.