Optical Rotatory Power of 2,4-Dimethyl-4-ethylheptane, a Tetraalkylmethane

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Optical rotatory power has never been determined for aliphatic unsymmetrical tetraalkylmethanes; indeed, hydrocarbons in which a central carbon has attached four different alkyl groups have been prepared only recently.[1,2] The recent resolution[3] of a trialkylacetic acid, 2,4-dimethyl-2-ethylpentanoic acid, has rendered feasible the preparation of individual enantiomers of some such quaternary hydrocarbons. The preparation of 2,4-dimethyl-4-ethylheptane of 25% optical purity has been carried out.

(−)Acid chloride (25% optically pure) was converted to (−)2,4-dimethyl-4-ethylheptan-5-one by treatment with diethylcadmium in the usual way. Several varied attempts to prepare a semicarbazone or 2,4-dinitrophenylhydrazone derivative failed, undoubtedly because of the extreme steric hindrance about the carbonyl group easily demonstrated by models. Reduction of the carbonyl group by various published modifications of the Wolff–Kishner reaction[4] also failed for the reason that the hydrazone was not formed. The reduction was finally successful when run in anhydrous homogeneous solution. This modified Wolff–Kishner reaction was carried out in decanol containing dissolved potassium hydroxide and anhydrous hydrazine and might be a general method for the reduction of very hindered carbonyl groups.

The resulting quaternary hydrocarbon had an infrared spectrum quite similar to those of typical aliphatic hydrocarbons of comparable size. In particular, a careful comparison was made to the spectrum of decane which could be a by-product through a possible though implausible oxidation–reduction reaction involving the solvent decanol. The several small but distinct differences in the spectra showed that the 2,4-dimethyl-4-ethylheptane could be contaminated by decane to only a minor extent, if any.

Since the asymmetric carbon is quaternary, racemization cannot reasonably have occurred during the reactions; yet, the optical activity found for the hydrocarbon, $[\alpha]_D$ −0.008 ± 0.004°, differs insignificantly from zero. It is clear that compounds of this type possess very little optical rotatory power. It is interesting to note in this connection that if the measured optical activity is taken at face value, the result for the optically pure hydrocarbon, $[\alpha]_D$ −0.03 ± 0.015°, $[M]_D$ −0.05 ± 0.03°, compares quite well with the value, $[M]_D$ 0.055°, predicted by the empirical method of Thomson.[5]

(1) *Cf.* R. Ya. Levina and N. P. Shusherina, *J. Gen. Chem.* (*U. S.S.R.*), **22**, 577 (1952); *C. A.*, **47**, 2679 (1953); N. Rabjohn and M. J. Latina, THIS JOURNAL, **76**, 1389 (1954).

(2) It should be noted, however, that unsymmetrical quaternary hydrocarbons containing a cyclohexyl group have been known for forty years [O. M. Halse, *J. prakt. Chem.*, **92**, 44 (1915)].

(3) W. E. Doering and K. B. Wiberg, THIS JOURNAL, **72**, 2608 (1950).

(4) Huang-Minlon, *ibid.*, **68**, 2487 (1946); C. H. Herr, F. C. Whitmore and R. W. Schiessler, *ibid.*, **67**, 2061 (1945).

(5) T. R. Thomson, *ibid.*, **75**, 6070 (1953).

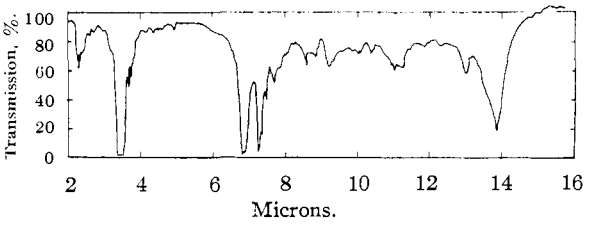

Fig. 1.—Infrared spectrum of 2,4-dimethyl-4-ethylheptane; cell thickness 0.095 cm.

Experimental

2,4-Dimethyl-4-ethylheptan-5-one.—A solution of diethylcadmium in benzene was prepared in the usual way[6] from 13.8 g. of magnesium, 67.8 g. of ethyl bromide and 57.2 g. of anhydrous cadmium chloride. Twenty grams of methylethylisobutylacetyl chloride in benzene was added and the mixture was refluxed for four hours. After decomposition with ice and hydrochloric acid, the benzene layer was separated, washed and dried. Distillation gave 18.0 g. (89%) of the ketone, b.p. 199–202°, having $n^{25}D$ 1.4347 and d^{25}_4 0.8420. The carbonyl stretching band at 5.88 μ is normal for aliphatic ketones.

Anal. Calcd. for $C_{11}H_{22}O$: C, 77.6; H, 13.0. Found: C, 77.3; H, 12.9.

When the preparation was repeated with 9.5 g. of optically active methylethylisobutylacetyl chloride, $\alpha^{23.5}D$ −4.38° (*l* 1, neat),[7] there was obtained 5.8 g. (63%) of ketone, b.p. 196–200°, having $n^{25}D$ 1.4330 and $\alpha^{22}D$ −4.58° (*l* 1, neat). The infrared spectrum was superimposible on that of the racemic ketone.

2,4-Dimethyl-4-ethylheptane.—Fifty-six milliliters of decanol, 7.8 g. of potassium hydroxide, 6.4 g. of the optically active ketone and 3.25 ml. of anhydrous hydrazine[8] were refluxed for two hours using a variable take-off head. Refluxing was continued during the next three hours with slow removal of the water using the take-off head. The bath temperature then was raised slowly to 235–240° and maintained at this point for four hours while the stoichiometric amount of nitrogen was evolved.

At the end of the reaction the mixture was steam distilled several times, since the hydrocarbon steam distils faster than the decanol. The resulting product was fractionated through a small column and finally refluxed with and distilled from potassium yielding 1.7 g. (33%) of hydrocarbon, b.p. 167–169°, having $n^{20}D$ 1.4141, d^{25}_4 0.7326, $[\alpha]^{22}D$ −0.060 ± 0.004°, $[\alpha]^{24}_{5461}$ −0.070 ± 0.006°.

Anal. Calcd. for $C_{11}H_{24}$: C, 84.5; H, 15.5. Found: C, 84.7; H, 15.6.

However, the infrared spectrum taken on a thick film of the liquid showed the presence of 1% of ketone which is sufficient to impart the optical activity found. The hydrocarbon, cooled to 0°, was shaken with an equal volume of precooled sulfuric acid for less than one minute. The hydrocarbon layer was drawn off and washed with water. After drying with anhydrous potassium carbonate it was refluxed with and distilled from potassium. Although the hydrocarbon contains a tertiary hydrogen, the conditions of the sulfuric acid wash were too mild to cause appreciable reaction.[9] The hydrocarbon had $n^{20}D$ 1.4142 and $d^{25.5}_4$ 0.7297,[10] and infrared spectrum was unchanged except that all trace of the carbonyl band at 5.88 microns had vanished. Determinations of the optical activity gave the results: αD −0.018 ± 0.005°, −0.005 ± 0.008°, −0.010 ± 0.004°; α_{5461} −0.015 ± 0.005°, +0.001 ± 0.006° (*l* 2, neat).

(6) J. Cason and H. Rapoport, "Laboratory Text in Organic Chemistry," Prentice–Hall, Inc., New York, N. Y., 1950, p. 329.

(7) This material, generously donated by Professors W. E. Doering and K. Wiberg, had been prepared from methylethylisobutylacetic acid, $[\alpha]^{25}D$ −3.3°, corresponding to 25% optical purity.

(8) L. I. Smith and K. L. Howard, *Org. Syntheses*, **24**, 53 (1944).

(9) R. L. Burwell, L. G. Maury and R. B. Scott, THIS JOURNAL, **76**, 5828 (1954).

(10) A. W. Francis, *Ind. Eng. Chem.*, **35**, 448 (1943), calculates by empirical methods b.p. 178.9°, d^{20}_4 0.7550 and $n^{20}D$ 1.4244. Using these values, the predicted $[R]_D$ value, 61.8, is in poor agreement with the calculated value, 53.1. The value found for the hydrocarbon is 53.4.

Racemic (*dl*)-hydrocarbon prepared in the same way had b.p. 168–170°, $n^{25}D$ 1.4130, m.p. −26.5°, and the same infrared spectrum.

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The Stability of N-Ethylmaleimide and its Reaction with Sulfhydryl Groups

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N-Ethylmaleimide (NEM) has been shown to react rapidly and specifically with sulfhydryl groups,[1] to be useful in the titration of such groups in proteins,[2] and to be an antimitotic agent.[1] In view of its potential application to the study of biochemical reactions involving sulfhydryl groups, we have made some observations on the rate of reaction and the stability of NEM.

Changes in the absorption spectrum of NEM on reaction with glutathione (GSH), in the region of 205–240 mμ, have been observed by Friedmann.[3] He shows also the broad peak centered at 302 mμ (molar extinction coefficient, ϵ_M 620), but does not mention its behavior in this reaction. The virtually complete disappearance of this peak, which takes place on combination with mercaptans, or on decomposition of NEM, has been used here to follow the rate of reaction and the instability of the compound at various *p*H values.

For 1.25×10^{-3} *M* solutions of NEM in water or 0.05 *M* buffers, the change in optical density with time at 302 mμ is shown in Fig. 1, I. The first-order reaction taking place at *p*H values above neutrality is probably a hydrolysis of the imide linkage since the resulting product combines with mercaptans only very slowly and has an absorption spectrum indistinguishable from that of synthetic N-ethylmaleamic acid. This instability should be taken into account in any quantitative procedure using NEM, such as the titration of sulfhydryl groups,[2] in which there is any possibility of the reagent being exposed to alkaline conditions.

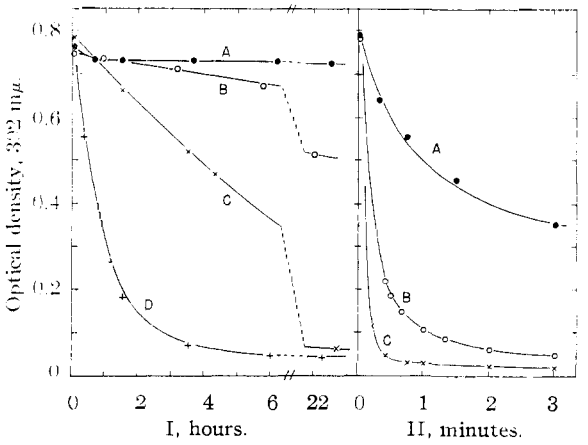

Fig. 1.—I, rate of decomposition of 1.25×10^{-3} *M* NEM in water or 0.05 *M* potassium acetate, *p*H 5.0 (A); potassium phosphate, *p*H 7.0 (B); tris-(hydroxymethyl)-aminomethane, *p*H 8.0 (C); 2-amino-2-methyl-1,3-propanediol, *p*H 9.0 (D). II, rate of reaction of NEM with GSH (both 10^{-3} *M*) in 0.1 *M* sodium acetate, *p*H 5.0 (A); potassium phosphate, *p*H 6.1 (B), and *p*H 7.0 (C).

The rate of reaction of NEM with GSH as a function of *p*H is shown in Fig. 1, II. Clearly, at about neutrality, the reaction with GSH is so much faster than the decomposition of the reagent that it may be used for quantitative purposes. The absolute change in molar extinction coefficient ($\Delta\epsilon_M$ 0.61 × 10^3), however, is only one-twelfth of that observed by Boyer[4] at 250 mμ when *p*-mercuribenzoate reacts with mercaptans, ($\Delta\epsilon_M$ 7.6 × 10^3), and in general it is probably not sensitive enough for photometric titration of protein sulfhydryl groups.

On the basis of this optical method, there is no evidence of appreciable reaction of NEM at *p*H 7 with S-acetyl GSH, diacetyl 2-mercaptoethylamine, oxidized glutathione, ethanol, ethylamine or HCN. Cysteine and H_2S react rapidly, as does potassium borohydride at *p*H 9. For comparison, some determinations have been made of the rate of combination of NEM with myokinase as assessed by inactivation of the enzyme. At *p*H 7.5 maximal inactivation (95%) was not reached until after treatment for 30 minutes with 2×10^{-4} *M* NEM. Excess NEM must have been present, since at *p*H 9 as little as 5×10^{-5} *M* NEM gave the same inactivation in 5 minutes. The time curve of inactivation was smooth and showed no evidence for classes of sulfhydryl groups having different reactivities.[5]

The situation with this enzyme is not clear, however, since the original preparation was activated fivefold when assayed in the presence of GSH; yet in the original state it was susceptible to almost complete inactivation by NEM. The GSH activation is thus not explainable simply by the existence of sulhydryl groups as disulfides, in which condition they should be immune to the inhibitor.

Experimental

Spectra were obtained in a Cary Recording Spectrophotometer, and reactions were followed in a Beckman Model DU Spectrophotometer.

N-Ethylmaleamic acid was synthesized from maleic anhydride and ethylamine as described by Piutti.[6]

Myokinase was prepared from horse muscle, essentially by the procedure of Colowick and Kalckar[7] with the addition of a fractionation between 17.5 and 35% saturated ammonium sulfate. The enzyme was assayed in a system containing 200 μ*M* tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.5, 5 μ*M* magnesium chloride, 2.5 μ*M* adenosine diphosphate, 20 μ*M* GSH, 30 μ*M* glucose, and excess hexokinase in 1.0 ml., by measuring loss of acid-labile phosphate. Of the above preparation, 3 μg. of protein transferred 1.0

(1) E. Friedmann, D. H. Marrian and I. Simon Reuss, *Brit. J. Pharmacol.*, **4**, 105 (1949); *Biochim. Biophys. Acta*, **9**, 61 (1952); E. Friedmann, *Bull. soc. chim. biol.*, **31**, 506 (1949).

(2) T.-C. Tsao and K. Bailey, *Biochim. Biophys. Acta*, **11**, 102 (1953).

(3) E. Friedmann, *ibid.*, **9**, 65 (1952).

(4) P. D. Boyer, THIS JOURNAL, **76**, 4331 (1954).

(5) E. S. G. Barron, *Advances in Enzymol.*, **11**, 201 (1951).

(6) A. Piutti and E. Giustiniani, *Gazz. chim. ital.*, **26**, 431 (1896).

(7) S. P. Colowick and H. M. Kalckar, *J. Biol. Chem.*, **148**, 117 (1943).