Anal. Calcd. for $C_{14}H_{20}$: C, 89.29; H, 10.71. Found: C, 89.71; H, 10.28.

1-Phenyl-1-cycloöctene (XVI).—Pure cycloöctanone, b.p. 113–113.5° (60 mm.), was regenerated from its semicarbazone.[14] A solution of phenylmagnesium bromide was prepared from 17.3 g. of bromobenzene and 2.67 g. of magnesium turnings in 75 ml. of dry ether. A solution of 12.6 g. of cycloöctanone in 75 ml. of dry ether was added with stirring during 30 minutes at a rate which maintained reflux. The mixture was stirred and heated under reflux for an additional period of 30 minutes, and then was cooled in ice. A solution of 50 g. of ammonium chloride in 150 ml. of water was added, slowly at first, and the mixture was stirred at room temperature for four hours and allowed to stand overnight. The ether layer was separated, the aqueous layer was extracted with 125 ml. of ether, and the combined ethereal solutions were dried over sodium sulfate. The ether was distilled, and the residue was dissolved in 150 ml. of benzene and heated in a bath at 100–110° for five hours. This treatment did not result in complete dehydration of the tertiary alcohol, for concentration and distillation yielded 4.1 g. (32%) of recovered cycloöctanone and 10.74 g. of a mixture of 1-phenyl-1-cycloöctanol and 1-phenyl-1-cyclooctene, b.p. 94.5–107° (0.55–1.5 mm.), n^{25}_{D} 1.5582–1.5608. A 5.71-g. portion of this mixture was converted to pure 1-phenyl-1-cycloöctene by heating a solution in 100 ml. of benzene with 50 mg. of iodine under reflux in a flask attached to a Dean and Stark water separator[15] for three hours. The benzene solution was washed with sodium thiosulfate solution to remove iodine, and the light yellow product obtained by distillation under reduced pressure was rewashed with sodium thiosulfate and redistilled through a semi-micro column.[8] The yield of 1-phenyl-1-cycloöctene[16] (XVI) was 3.57 g., b.p. 82–83.5° (0.2 mm.), n^{25}_{D} 1.5612, d^{25}_{4} 0.9781; M_D calcd. 60.58, found 61.69 (exaltation 1.11).

Anal. Calcd. for $C_{14}H_{18}$: C, 90.26; H, 9.74. Found: C, 90.59; H, 9.83.

Phenylcycloöctane (XVII).—An authentic sample of phenylcycloöctane was prepared by hydrogenating a solution of 2.24 g. of 1-phenyl-1-cycloöctene in 40 ml. of glacial acetic acid in the presence of 0.4 g. of 10% palladium-on-carbon.[11] The reduction was complete in 20 minutes and required 102.9% of one molar equivalent of hydrogen. The catalyst was separated by filtration, and the filtrate was concentrated under reduced pressure. A solution of the residue in ether was washed with 10% sodium carbonate solution and water, and was dried over magnesium sulfate. The extracts were concentrated, and the residue was distilled through a semi-micro column, yielding 1.88 g. (83%) of phenylcycloöctane as a colorless liquid, b.p. 86–87.5° (0.29 mm.) m.p. 7.3–8.5°, n^{25}_{D} 1.5299, d^{25}_{4} 0.9525; M_D calcd. 61.05, found 61.05.

Anal. Calcd. for $C_{14}H_{20}$: C, 89.29; H, 10.71. Found: C, 89.36; H, 10.71.

Ultraviolet Absorption Spectra.—The ultraviolet absorption spectra shown in Figs. 1 and 3 were determined for solutions of the compounds in purified cyclohexane[17] with a Beckman model DU quartz ultraviolet spectrophotometer. The following maxima were observed: In Fig. 1: Curve 1 (mixture of IX and XI), $\lambda_{max.1}$ 226 mμ (log ϵ 4.21), $\lambda_{max.2}$ 259 mμ (log ϵ 3.92); Curve 2 (mixture of IX and XI), $\lambda_{max.1}$ 229 mμ (log ϵ 4.17), $\lambda_{max.2}$ 254 mμ (log ϵ 4.10); Curve 3 (mixture of IX and XI, $\lambda_{max.1}$ 232 mμ (log ϵ 4.11), $\lambda_{max.2}$ 254 mμ (log ϵ 4.15); Curve 4 (XI), $\lambda_{max.}$ 252 mμ (log ϵ 4.27). In Fig. 3: Curve 1 (XIV), $\lambda_{max.1}$ 231 mμ (log ϵ 4.23), $\lambda_{max.2}$ 284 mμ (log ϵ 3.99); XIV prepared by thermal decomposition of the methiodide XII (not shown), $\lambda_{max.1}$ 232 mμ (log ϵ 4.20), $\lambda_{max.2}$ 284 mμ (log ϵ 4.00); Curve 2 (XV), $\lambda_{max.1}$ 232 mμ (log ϵ 4.23), $\lambda_{max.2}$ 284 mμ (log ϵ 3.94); Curve 3 (XVI), $\lambda_{max.}$ 248 mμ (log ϵ 4.07).

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(14) Prepared by L. L. Estes, Jr., and regenerated by the method of E. P. Kohler, M. Tishler, H. Potter and H. T. Thompson, THIS JOURNAL, **61**, 1061 (1939).

(15) E. W. Dean and D. D. Stark, *Ind. Eng. Chem.*, **12**, 486 (1920).

(16) L. F. Fieser and J. Szmuszkovicz, THIS JOURNAL, **70**, 3352 (1948), have prepared a mixture of 1-phenyl-1-cycloöctanol and 1-phenyl-1-cycloöctene in a similar manner but did not isolate the pure olefin.

(17) M. E. Maclean, P. J. Jencks and S. F. Acree, *J. Research Natl. Bur. Standards*, **34**, 271 (1945).

CAMBRIDGE, MASS. RECEIVED OCTOBER 25, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Cyclic Polyolefins. XVII. Preparation of Arylcycloöctatetraenes from Cycloöctatetraene and Organometallic Compounds[1]

BY ARTHUR C. COPE AND MARK R. KINTER[2]

The reaction of phenyllithium with cycloöctatetraene has been found to proceed by addition, followed by a process equivalent to the transfer of lithium hydride from the addition compound to another molecule of cycloöctatetraene. The products isolated after hydrolysis were phenylcycloöctatetraene, phenylcycloöctatriene, a mixture of 1,3,5- and 1,3,6-cycloöctatrienes and recovered cycloöctatetraene. Phenylsodium reacted in the same way, yielding phenylcycloöctatetraene and cyclooctatrienes. Likewise *p*-dimethylaminophenyllithium yielded *p*-dimethylaminophenylcycloöctatetraene and cycloöctatrienes. The reaction of phenylmagnesium bromide with cycloöctatetraene resulted in the formation of biphenyl- and cyclooctatrienes.

Phenylcycloöctatetraene has been characterized by the preparation of a 1:1 complex with silver nitrate, addition compounds with maleic anhydride and *p*-benzoquinone, and by quantitative hydrogenation to phenylcycloöctane. *p*-Dimethylaminophenylcycloöctatetraene was reduced quantitatively to *p*-dimethylaminophenylcycloöctane. A crystalline salt was obtained from *p*-dimethylaminophenylcycloöctatetraene and *d*-camphor-10-sulfonic acid, but recrystallization of the salt failed to result in the separation of diastereomeric forms.

In a recent communication[3] the reactions of phenyllithium and *p*-dimethylaminophenyllithium with cycloöctatetraene were reported to form phenylcycloöctatetraene (I) and *p*-dimethylaminophenylcycloöctatetraene (IV), respectively. This paper presents additional information concerning these unusual reactions and the properties of I and IV, and in addition describes the reactions of cycloöctatetraene with phenylsodium and phenylmagnesium bromide.

Organolithium compounds are known to add to the carbon–carbon double bonds of aryl-substituted olefins[4] and fulvenes,[5] and to conjugated dienes in the initiation of polymerization.[6] A study of the reaction of organolithium compounds with cycloöctatetraene accordingly was undertaken, in order to determine whether substituted

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(1) Supported in part by a du Pont grant in aid of fundamental research, and in part by the Office of Naval Research under Contract N5ori-07822, Project Designation NR-055-96.

(2) du Pont Fellow, 1950–1951.

(3) A. C. Cope and M. R. Kinter, THIS JOURNAL, **72**, 630 (1950).

(4) K. Ziegler, F. Crössmann, H. Kleiner and O. Schäfer, *Ann.*, **473**, 1 (1929).

(5) K. Ziegler and W. Schäfer, *ibid.*, **511**, 101 (1934).

(6) K. Ziegler, *Angew. Chem.*, **49**, 499 (1936).

cycloöctatrienes (of interest as possible intermediates in the synthesis of substituted cycloöctatetraenes) were formed. Phenyllithium was investigated first because the properties of phenylcycloöctatriene were known.[7]

Phenyllithium reacted slowly with cycloöctatetraene in ether solution, or more rapidly if the ether was distilled and the mixture was heated at 90°, to give a mixture of organolithium compounds that was decomposed by hydrolysis. Distillation of the product separated a low boiling fraction containing cycloöctatrienes and recovered cycloöctatetraene from a high boiling fraction composed principally of phenylcycloöctatetraene and phenylcycloöctatrienes. The phenylcycloöctatetraene was purified by conversion into a crystalline 1:1 complex with silver nitrate, from which the hydrocarbon was regenerated by treatment with ammonium hydroxide, and cycloöctatetraene was separated from cycloöctatrienes by extraction with aqueous silver nitrate.[8] The yields of these products (based upon equations 1 and 2 below) were: phenylcycloöctatetraene (I), 27%; phenylcyclooctatriene (II), 9%; recovered cycloöctatetraene, 51% according to colorimetric analysis and 50% by isolation; and a mixture of 1,3,6- and 1,3,5-cycloöctatrienes (IIIa and IIIb), 36% according to colorimetric analysis and 20% by isolation. The analytical figures show that I and III were formed in approximately equimolar amounts, as required by equations 1 and 2. Small amounts of bromobenzene and biphenyl resulting from incomplete reaction and coupling in the preparation of phenyllithium also were isolated.

Phenylcycloöctatetraene (I) was isolated through its crystalline, yellow-green complex with silver nitrate as an orange-yellow liquid which polymerized on standing in contact with the air, but was stable on storage in a nitrogen atmosphere at 0° for several months. The hydrocarbon I was characterized by its ultraviolet (Fig. 1) and infrared (Fig. 2) absorption spectra, by quantitative hydrogenation to phenylcycloöctane (identical with an authentic sample[7]), and by preparation of a crystalline 1:1 complex with silver nitrate and crystalline addition compounds with maleic anhydride and *p*-benzoquinone. The analyses of I and the three derivatives prepared from it, the fact that four molar equivalents of hydrogen were absorbed in the quantitative reduction forming phenylcycloöctane, and comparison of the colors of I and cycloöctatetraene provide convincing evidence for the structure of phenylcycloöctatetraene. The high boiling reaction product which failed to react with silver nitrate proved to be a mixture of phenylcycloöctatrienes, probably contaminated with phenylbicycloöctadienes. Evidence supporting this conclusion was provided by analysis, absorption of 86% of three molar equivalents of hydrogen and formation of impure phenylcycloöctane in a quantitative reduction, and by comparison of the ultraviolet absorption spectrum of II with the spectrum of phenylcycloöctatriene prepared from pseudopelletierine.[7]

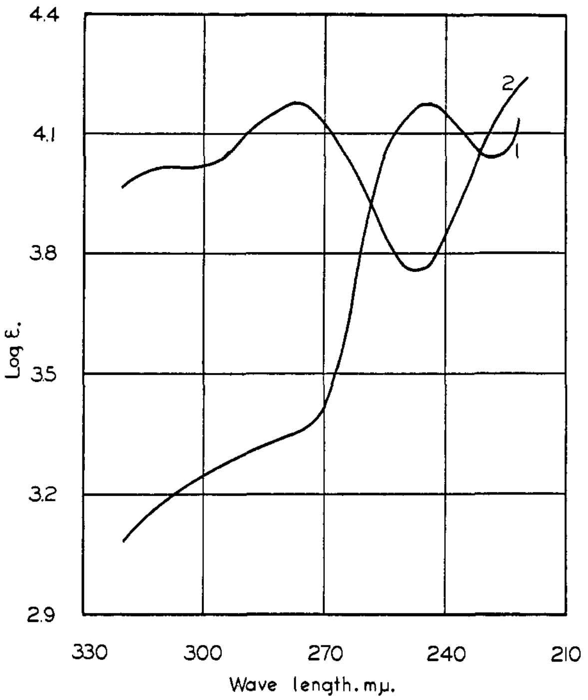

Fig. 1.—Ultraviolet absorption spectra, determined with a Beckman model DU quartz ultraviolet spectrophotometer: curve 1, phenylcycloöctatetraene (I); curve 2, *p*-dimethylaminophenylcycloöctatetraene (IV), both in cyclohexane solution.

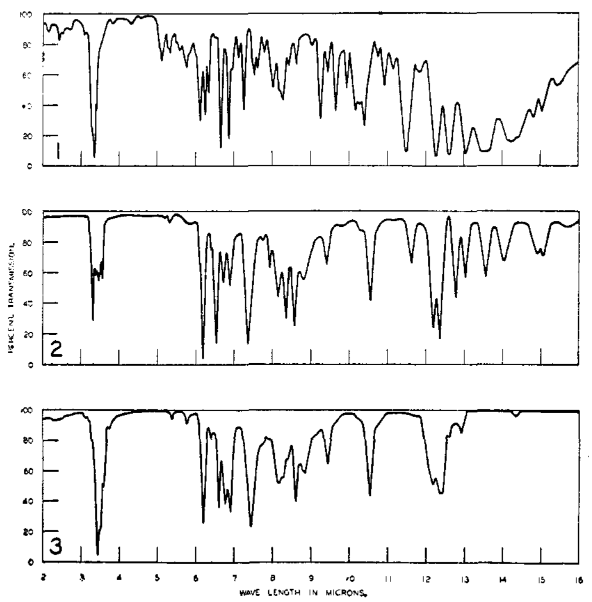

Fig. 2.—Infrared absorption spectra: curve 1, phenylcycloöctatetraene (I) (pure liquid); curve 2, *p*-dimethylaminophenylcycloöctatetraene (IV), spectrum of a solution (100 mg./ml.) in carbon tetrachloride in the region 2–7.4μ and in carbon disulfide in the region 7.4–16μ; curve 3, *p*-dimethylaminophenylcycloöctane (VI), spectrum of a solution (100 mg./ml.) in carbon tetrachloride in the region 2–7.4μ and in carbon disulfide in the region 7.4–16 μ (sample prepared by hydrogenation of IV).

The colorless hydrocarbon remaining after separation of cycloöctatetraene from the low boiling

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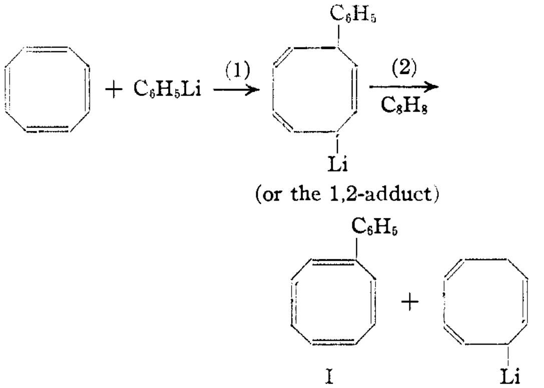
(7) A. C. Cope and A. A. D'Addieco, THIS JOURNAL, **73**, 3419 (1951).

(8) A. C. Cope and F. A. Hochstein, *ibid.*, **72**, 2515 (1950).

hydrocarbon fraction with aqueous silver nitrate was a mixture of 1,3,6- and 1,3,5-cycloöctatrienes containing 7% of bromobenzene (determined by quantitative analysis for bromine). The nature of the mixture of cycloöctatrienes was determined by comparing its ultraviolet absorption spectrum with the spectra of the two isomers, and by treating the mixture with potassium *t*-butoxide to convert the 1,3,6-cycloöctatriene into 1,3,5-cycloöctatriene.[8] The identity of the resulting 1,3,5-cycloöctatriene was confirmed by its ultraviolet absorption spectrum and by the melting points of its silver nitrate complex[8] and maleic anhydride adduct.[9]

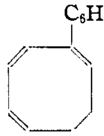
Additional information concerning the reaction of phenyllithium with cycloöctatetraene was obtained by decomposing the intermediate organolithium compounds by carbonation rather than by hydrolysis. The amount of phenylcycloöctatetraene in the product was essentially unchanged, but the low boiling hydrocarbon fraction did not contain cyclooctatrienes; instead an acid was formed which polymerized during attempted isolation. Accordingly the following reactions occur when cycloöctatetraene is treated with phenyllithium.



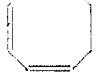
I

(or the 1,2-adduct)

Hydrolysis of the organolithium compounds formed in reactions (1) and (2) would produce the other products which were isolated, II and III.



II



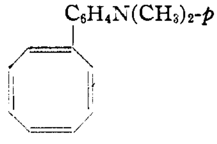
IIIa

IIIb

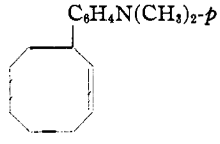
(or other isomers
in which the double
bonds are conjugated)

The reaction of cycloöctatetraene with phenylsodium followed a course similar to the reaction with phenyllithium. The products which were isolated were phenylcycloöctatetraene (22%), a mixture of 1,3,6- and 1,3,5-cycloöctatrienes (23%) and recovered cycloöctatetraene (32%).

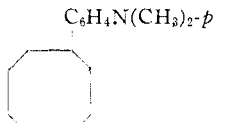
The reaction of cycloöctatetraene with *p*-dimethylaminophenyllithium was conducted in the same manner as the reaction with phenyllithium, and the products were separated into basic and neutral fractions by extraction with dilute hydrochloric acid. The neutral fraction yielded 36% of a mixture of 1,3,6- and 1,3,5-cycloöctatrienes and 42% of recovered cycloöctatetraene. Distillation of the basic products obtained by making the acid extracts alkaline separated N,N-dimethylaniline (formed by hydrolysis of *p*-dimethylaminophenyllithium) and *p*-dimethylaminophenylcyclooctatetraene (IV), which was obtained in 27% yield as orange leaflets melting at 90–90.5° by crystallization from methanol. *p*-Dimethylaminophenylcycloöctatetraene was characterized by its ultraviolet (Fig. 1) and infrared (Fig. 2) absorption spectra. It decomposed, apparently because of oxidation, upon treatment with silver nitrate and with *p*-benzoquinone, and failed to form a crystalline adduct with maleic anhydride. Evidence for the structure of IV was obtained by analysis and by analysis of its picrate, and by a quantitative hydrogenation in which 99% of four molar equivalents of hydrogen was absorbed. The reduction product was identified as *p*-dimethylaminophenylcycloöctane (VI) by preparation of the picrate, m.p. 149–150°, which was compared with a known sample. The authentic sample of *p*-dimethylaminophenylcycloöctane was prepared by first coupling *p*-dimethylaminophenyllithium with 3-bromocycloöctene to form 3-*p*-dimethylaminophenylcycloöctene (V), the position of the double bond was not proved and rearrangement into conjugation with the aromatic nucleus may have occurred), followed by hydrogenation of V to VI. The *p*-dimethylaminophenylcycloöctane prepared in this way had the same infrared spectrum within experimental error as the sample obtained by reduction of IV (Fig. 2), and the picrates prepared from the two samples had the same melting points and mixed melting point.



IV



V



VI

p-Dimethylaminophenylcycloöctatetraene reacted with *d*-camphor-10-sulfonic acid in ether containing small amounts of water and ethanol to form a salt which crystallized as a dihydrate, m.p. 56–57°. Three recrystallizations of this salt failed to change its melting point or optical rotation, and the base which was regenerated from the recrystallized salt by hydrolysis with water was optically inactive. This result indicates either that the structure of IV is not asymmetric, or that the *d*-camphor-10-sulfonic acid salt is not suitable for the resolution under the conditions investigated. Mono-substituted cycloöctatetraenes would be optically inactive because of rapid racemization if the non-planar cycloöctatetraene

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[9] A. C. Cope, H. R. Nace and L. L. Estes, Jr., THIS JOURNAL, **72**, 1127 (1950).

ring (whether boat, crown or chair[10]) were readily deformed, as for example, by assuming a planar configuration through bending of the carbon–carbon bonds. In that event monosubstituted cycloöctatetraenes could not be resolved just as simple unsymmetrical tertiary amines cannot because of carbon–nitrogen bond motions which bring the three substituents into the same plane as the nitrogen atom.[11] Further attempts to resolve a substituted cycloöctatetraene will be made with a compound which forms more stable salts than IV, which is very weakly basic.

The reaction of phenylmagnesium bromide with cycloöctatetraene in ether at 100°, followed by hydrolysis, formed biphenyl (45%) and a mixture of 1,3,6- and 1,3,5-cycloöctatrienes (42%). Presumably the initial products are biphenyl and di-(bromomagnesium) derivatives of cycloöctatriene, from which IIIa and IIIb are formed on hydrolysis. Further evidence that the cycloöctatetraene is responsible for the formation of biphenyl in this reaction was obtained by a control experiment, in which ethylcyclohexane was substituted for cycloöctatetraene. The quantity of biphenyl isolated (6.6%) was an amount to be expected as a by-product from coupling in preparation of the Grignard reagent.

Experimental[12]

Reaction of Phenyllithium with Cycloöctatetraene.—A dry 1-l. three-necked flask was equipped with a mechanical stirrer, a dropping funnel and a reflux condenser, and the funnel and condenser were protected with drying tubes attached to a source of nitrogen under a pressure of 1–2 cm. The system was flushed with nitrogen, and 300 ml. of dry ether and 7.65 g. (1.1 gram atoms) of $^1/_8$ in. lithium wire (cut into 1-cm. pieces) were placed in the flask. A solution of 78.5 g. (0.5 mole) of bromobenzene in 100 ml. of dry ether was added with stirring during 70 minutes at a rate that maintained reflux, and the mixture was heated under reflux for an additional period of one hour. Cycloöctatetraene[13] (104 g., 1.0 mole) was added, and the reflux condenser was replaced by a 20 × 1.5-cm. Vigreux column. The mixture was heated gradually to a bath temperature of 90° during 1 hour, while most of the ether distilled, and then was stirred at that temperature for 2 hours. The orange colored mixture was cooled in ice, and the dry ether which had been removed by distillation was returned to the flask. Water (500 ml.) was added slowly with stirring to decompose the organolithium compounds present, and the red ether layer was separated, washed three times with water, and dried over magnesium sulfate. Two distillations of the low boiling material through a 20 × 1.5-cm. helix-packed column separated the ether solvent and benzene formed by hydrolysis of phenyllithium from fraction 1 (described below, 72.9 g., b.p. 74–77° at 97 mm., $n^{25}\text{D}$ 1.5295) and a high boiling residue. Distillation of the residue through a semi-micro column[14] separated 37.3 g. of a mixture of phenylcycloöctatetraene and phenylcycloöctatriene (fraction 2), b.p. 94–95° (0.3 mm.), $n^{25}\text{D}$ 1.6168, from 8.7 g. of a red, high-boiling residue.

Phenylcycloöctatetraene (I) was isolated as the silver nitrate complex by treating a solution of fraction 2 in 300 ml. of boiling absolute ethanol with 35.2 g. of finely powdered silver nitrate. After heating for 10 minutes, all of the silver nitrate dissolved forming a yellow-green solution, and on cooling to 0° a yellow-green, crystalline silver nitrate complex separated. The crystals were collected on a filter, washed with two 50-ml. portions of cold ether, and air-dried for one-half hour (weight 45.5 g.). (The filtrate contained phenylcycloöctatriene, which was isolated by a procedure described below.) The silver nitrate complex was decomposed by shaking with a solution of 100 ml. of concentrated ammonium hydroxide in 100 ml. of water. The orange liquid which was formed was extracted with two 50-ml. portions of ether, and the combined extracts were washed with water, dried over magnesium sulfate, and concentrated. Distillation through a semi-micro column[14] yielded 22.6 g. (25%) of I as an orange liquid, b.p. 94–95° (0.3 mm.), $n^{25}\text{D}$ 1.6181, d^{25}_4 1.0335.

Anal. Calcd. for $C_{14}H_{12}$: C, 93.29; H, 6.71. Found: C, 93.24; H, 6.83.

Hydrogenation of a solution of 1.415 g. of I in 50 ml. of methanol in the presence of 0.3 g. of 10% palladium-on-carbon[15] at atmospheric pressure and room temperature was complete in thirty-five minutes and required 99% of four molar equivalents of hydrogen. After separation of the catalyst, distillation through a semi-micro column yielded 1.18 g. (80%) of phenylcycloöctane, b.p. 108–111° (1.1 mm.), $n^{25}\text{D}$ 1.5302, m.p. and mixed m.p. with an authentic sample,[7] 7–8°.

Phenylcycloöctatetraene–silver nitrate complex was prepared by adding 1.70 g. of powdered silver nitrate to a hot solution of 1.80 g. of I in 18 ml. of absolute ethanol. The mixture was heated on a steam-bath for 5 minutes until a clear yellow-green solution was formed, and then cooled in ice with stirring. The yellow-green crystals were collected on a filter, washed with 10 ml. of cold, dry ether, and air dried for one-half hour. The yield of the complex was 3.24 g. (93%). An analytical sample was recrystallized from absolute ethanol and dried at 0.5 mm. and room temperature for 2 hours; m.p. 144.5° (dec., introduced at 135° and heated 2° per minute).

Anal. Calcd. for $C_{14}H_{12}\cdot AgNO_3$: C, 48.02; H, 3.46. Found: C, 48.11; H, 3.54.

Phenylcycloöctatetraene–maleic anhydride adduct was prepared by heating a solution of 1.10 g. of I and 0.60 g. of freshly distilled maleic anhydride in 10 ml. of dry benzene under reflux for 15 hours. The pale yellow solution was concentrated to a volume of 5 ml. and cooled. The white crystalline maleic anhydride adduct which separated and a second crop obtained by concentrating the filtrate totaled 1.46 g. (91%), m.p. 171–172°. An analytical sample which was recrystallized from benzene melted at 173–174°.

Anal. Calcd. for $C_{18}H_{14}O_3$: C, 77.67; H, 5.07. Found: C, 77.69; H, 5.42.

Phenylcycloöctatetraene–*p*-benzoquinone adduct was prepared by heating a solution of 0.90 g. of I and 0.54 g. of *p*-benzoquinone in 15 ml. of benzene under reflux for 20 hours. The solution was concentrated to dryness under reduced pressure, and the yellow solid residue was dissolved in the minimum amount (10 ml.) of boiling 95% ethanol. On cooling in ice small yellow needles of the adduct formed, which after filtration and drying at 100° weighed 1.10 g. (76%), m.p. 189.5–190°. One recrystallization from a mixture of benzene and 95% ethanol furnished an analytical sample with a constant melting point of 192–192.5°.

Anal. Calcd. for $C_{20}H_{16}O_2$: C, 83.31; H, 5.59. Found: C, 83.41; H, 5.79.

Phenylcycloöctatriene (probably a mixture of isomers) was isolated from the alcohol filtrate and ether washings which were separated from the phenylcycloöctatetraene–silver nitrate complex prepared from fraction 2 (above) by the following procedure. The solution was concentrated under reduced pressure to a volume of 50 ml., and shaken with 50 ml. of concentrated ammonium hydroxide and 50 ml. of water. The oil which separated was extracted with two 25-ml. portions of pentane, and the combined extracts were washed with water and then extracted with six 25-ml. portions of 50% aqueous silver nitrate. Treatment of the extracts with an excess of ammonium hydroxide caused the

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(10) J. W. Copenhaver and M. Bigelow, "Acetylene and Carbon Monoxide Chemistry," Reinhold Publishing Corp., New York, N. Y., 1949, p. 184.

(11) R. L. Shriner, R. Adams and C. S. Marvel in H. Gilman, "Organic Chemistry," 2nd ed., Vol. I, John Wiley and Sons, New York, N. Y., 1943, p. 411.

(12) Melting points are corrected and boiling points are uncorrected. We are indebted to Mr. S. M. Nagy and his associates for analyses and for the infrared spectra, which were determined with a Baird Double Beam Infrared Recording Spectrometer, Model B, fitted with a sodium chloride prism.

(13) A. C. Cope and L. L. Estes, Jr., THIS JOURNAL, **72**, 1129 (1950).

(14) C. W. Gould, Jr., G. Holzman and C. Niemann, *Anal. Chem.*, **20**, 361 (1948).

(15) *Org. Syntheses*, **26**, 32 (1946).

separation of a small additional amount of slightly impure phenylcycloöctatetraene, which after extraction and distillation amounted to 1.6 g. (2%), b.p. 93–94° (0.3 mm.), $n^{25}\mathrm{D}$ 1.6155. The pentane solution remaining after extraction with silver nitrate was washed with water, concentrated, and the residue was distilled through a semi-micro column.[14] Approximately 1 g. of biphenyl was separated as the first fraction, followed by three fractions of phenylcycloöctatriene totaling 8.52 g. (9%), b.p. 90–92° (0.3 mm.), $n^{25}\mathrm{D}$ 1.6100–1.6174. The last fraction, $n^{25}\mathrm{D}$ 1.6174, was analyzed.

Anal. Calcd. for $C_{14}H_{14}$: C, 92.26; H, 7.74. Found: C, 92.43; H, 7.99.

Hydrogenation of a solution of 0.188 g. of this fraction in 10 ml. of methanol in the presence of 0.2 g. of 10% palladium-on-carbon[15] was complete in one-half hour and required 86% of three molar equivalents of hydrogen. Distillation of the product yielded 0.13 g. of impure phenylcyclooctane, m.p. −11 to 2.3° and mixed m.p. with an authentic sample (melting at 7.8–8.5°), −0.8 to +3.8°.

The remainder of the crude phenylcycloöctatriene was redistilled; b.p. 90–92° (0.2 mm.), $n^{25}\mathrm{D}$ 1.6126. The ultraviolet absorption spectrum of this product ($\lambda_{\text{max.1}}$ 230 mμ, ϵ 11,750; $\lambda_{\text{min.}}$ 254 mμ, ϵ 5,020; $\lambda_{\text{max.2}}$ 288 mμ, ϵ 6,580) was similar to the spectrum of phenylcycloöctatriene prepared from pseudopelletierine,[7] $n^{25}\mathrm{D}$ 1.6072 ($\lambda_{\text{max.1}}$ 232 mμ, ϵ 16,900; $\lambda_{\text{min.}}$ 252 mμ, ϵ 4,410; $\lambda_{\text{max.2}}$ 284 mμ, ϵ 8,700).

An attempt to obtain a more homogeneous phenylcyclooctatriene sample by isomerization with potassium *t*-butoxide in *t*-butanol at the reflux temperature (a procedure used to convert 1,3,6-cycloöctatriene to the 1,3,5-isomer[8]) was unsuccessful. The ultraviolet absorption spectrum of the hydrocarbon was changed by this treatment, but had not become constant after a reflux period of 47 hours.

The low-boiling fraction 1 (72.9 g.) described above obtained from the reaction of phenyllithium with cycloöctatetraene contained 73% (51% recovery) of cycloöctatetraene (estimated colorimetrically[16] by comparison with solutions of cycloöctatetraene in cyclohexane of known concentration), 1% of bromobenzene (determined by quantitative analysis for bromine) and 26% of cycloöctatrienes (by difference, equivalent to 19.0 g. or a 36% yield). The cyclooctatetraene was separated by extracting a solution of 70 g. of fraction 1 in 70 ml. of pentane with nine 100-ml. portions of 20% aqueous silver nitrate, which was the minimum amount that removed the yellow color from the pentane solution. Water was added when necessary during the extractions to dissolve any solid (the cycloöctatetraene–silver nitrate complex) that separated. The silver nitrate extracts were added to 300 ml. of concentrated ammonium hydroxide, and the yellow cycloöctatetraene which separated on shaking was extracted with ether and distilled through a 20 × 1.5-cm. helix-packed column; the recovery was 52.1 g. (50%), b.p. 73–74° (88 mm.), $n^{25}\mathrm{D}$ 1.5333. The colorless pentane solution was washed with water, dried over magnesium sulfate, and the pentane was distilled through a 20 × 1.5-cm. helix-packed column. The residue was fractionated through a semimicro column,[14] and yielded 10.5 g. (20%) of a mixture of cycloöctatrienes, b.p. 74–78° (93 mm.), $n^{25}\mathrm{D}$ 1.5158. The ultraviolet absorption spectrum of this fraction indicated that it was a mixture of 1,3,5- and 1,3,6-cycloöctatrienes ($\lambda_{\text{max.}}$ 262 mμ, ϵ 2110 and $\lambda_{\text{min.}}$ 232 mμ, ϵ 1550). A solution of 5.0 g. of this mixture in 15 ml. of *t*-butyl alcohol containing the potassium *t*-butoxide prepared from 0.2 g. of potassium was heated under reflux for 2 hours in a nitrogen atmosphere to isomerize the 1,3,6-cycloöctatriene present to 1,3,5-cycloöctatriene.[8] The solution was cooled, diluted with 25 ml. of pentane, washed with five portions of water to remove *t*-butyl alcohol, and dried over sodium sulfate. Fractionation through a semimicro column yielded 4.06 g. of crude 1,3,5-cycloöctatriene, b.p. 80–81° (90 mm.), $n^{25}\mathrm{D}$ 1.5210. The ultraviolet absorption spectrum of this fraction ($\lambda_{\text{max.}}$ 264 mμ, ϵ 3,260 compared to $\gamma_{\text{max.}}$ 265 mμ, ϵ 3,600 for pure 1,3,5-cycloöctatriene[8]) indicated that it contained 90% of 1,3,5-cycloöctatriene, and bromine analysis (3.68% bromine) showed that 7% of bromobenzene was present. The 1,3,5-cycloöctatriene was characterized by preparation of the maleic anhydride adduct, m.p. and mixed m.p. with a known sample,[9] 143.5–144°.

In one case the organolithium compounds present in the reaction mixture obtained by heating phenyllithium and cycloöctatetraene were carbonated rather than decomposed by hydrolysis, in order to determine which hydrocarbons were present as lithium derivatives. Cycloöctatetraene (20.8 g.) was added to a solution of phenyllithium prepared from 15.7 g. of bromobenzene, 1.52 g. of lithium and 75 ml. of ether, and the mixture was heated in a bath at 90° for 2 hours after distillation of the ether in the manner previously described. The mixture was cooled in an ice-bath, diluted with 50 ml. of dry ether, and forced onto several hundred grams of finely powdered Dry Ice. The transfer was completed by rinsing the flask with two 50-ml. portions of dry ether. After volatilization of the Dry Ice, 200 ml. of water and enough sodium hydroxide to make the mixture definitely basic to litmus were added. The ethereal layer was separated, washed with water, dried over magnesium sulfate, and the ether was distilled through a 20 × 1.5-cm. helix-packed column. Distillation of the residue through a semimicro column[14] gave: (1) 9.70 g., b.p. 74–76° (90 mm.), $n^{25}\mathrm{D}$ 1.5320; (2) 4.88 g., b.p. 92–95° (0.2 mm.), $n^{25}\mathrm{D}$ 1.6141. Redistillation of fraction (1) yielded 8.89 g. of cycloöctatetraene, b.p. 74–75° (87 mm.), $n^{25}\mathrm{D}$ 1.5349, which was 96% pure according to colorimetric analysis. Treatment of fraction (2) with 4.6 g. of powdered silver nitrate in 50 ml. of hot absolute ethanol yielded 7.5 g. of the phenylcycloöctatetraene–silver nitrate complex, which on decomposition with ammonium hydroxide yielded 3.54 g. (20%) of phenylcycloöctatetraene, b.p. 101–103° (1 mm.), $n^{25}\mathrm{D}$ 1.6184. These data show that the hydrocarbons formed in the reaction contained little if any cycloöctatriene. In preliminary experiments, no crystalline products could be isolated from the acids obtained by acidification of the alkaline aqueous extract of the reaction mixture after carbonation.

Reaction of Phenylsodium with Cycloöctatetraene.—Powdered sodium (5.75 g., 0.25 mole) and 75 ml. of dry benzene were placed in a dry 200-ml. three-necked flask equipped with a mechanical stirrer, a reflux condenser, a thermometer and a dropping funnel. The air in the system was displaced with nitrogen, and 11.2 g. (0.10 mole) of chlorobenzene was added during one hour with rapid stirring and cooling to maintain a reaction temperature of 35–40°.[17] The mixture was stirred at 30° for an additional period of 90 minutes and then 20.8 g. (0.20 mole) of cycloöctatetraene was added. The mixture was stirred and heated under reflux for 1 hour, and then was cooled in ice while 50 ml. of ethanol was added, followed by 75 ml. of water. Dilute hydrochloric acid was added until the mixture was neutral, and the benzene layer was separated. The aqueous layer was extracted with 50 ml. of ether, and the combined benzene and ethereal solutions were washed twice with water and dried over magnesium sulfate. The solvent was distilled through a 20 × 1.5-cm. helix-packed column, and the products were fractionated through a semi-micro column. The low and high boiling fractions were separated into their components by treatment with silver nitrate by procedures similar to those described for separating the products formed from cycloöctatetraene and phenyllithium.

A solution of 10.0 g. of fraction 1 (11.6 g., b.p. 75–77° at 94 mm., $n^{25}\mathrm{D}$ 1.5262) in 15 ml. of pentane was extracted with six 20-ml. portions of 20% aqueous silver nitrate. Treatment of the extracts with ammonium hydroxide yielded 6.7 g. (32% recovery) of cycloöctatetraene, b.p. 74–75° (93 mm.), $n^{25}\mathrm{D}$ 1.5320. Distillation of the pentane solution yielded 2.44 g. (23%) of a mixture of cycloöctatrienes, b.p. 77–79° (91 mm.), $n^{25}\mathrm{D}$ 1.5086. Isomerization with potassium *t*-butoxide in *t*-butyl alcohol[8] yielded 1.82 g. of 1,3,5-cycloöctatriene, b.p. 80–81° (90 mm.), $n^{25}\mathrm{D}$ 1.5187, $\lambda_{\text{max.}}$ 264 mμ (ϵ 3140); $\lambda_{\text{min.}}$ 226 mμ (ϵ 1345), m.p. of the maleic anhydride adduct and mixed m.p. with a known sample,[9] 143.5–145°.

Fraction 2, 5.43 g., b.p. 100–102° (1 mm.), $n^{25}\mathrm{D}$ 1.6137, was treated with 5.13 g. of powdered silver nitrate in 60 ml. of hot absolute ethanol, and yielded 9.2 g. of the crystalline phenylcycloöctatetraene–silver nitrate complex. Treatment with ammonium hydroxide yielded 3.3 g. (18%) of phenylcycloöctatetraene, b.p. 97–98° (0.6 mm.), $n^{25}\mathrm{D}$ 1.6188. The filtrate separated from the silver nitrate adduct yielded an additional 0.65 g. (4%) of phenylcyclo-

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[16] Using a Lumetron Model 400-A Photoelectric Colorimeter fitted with a blue filter.

[17] This preparation of phenylsodium is based upon one described by H. Gilman, H. A. Pacevitz and O. Baine, THIS JOURNAL, **62**, 1517 (1940), in which toluene was used as the solvent.

octatetraene, separated by extraction with silver nitrate and regenerated with ammonium hydroxide.

p-Dimethylaminophenylcycloöctatetraene (IV).—A solution of *p*-dimethylaminophenyllithium[18] was prepared from 20.0 g. (0.1 mole) of recrystallized *p*-bromodimethylaniline and 1.54 g. (0.22 gram atom) of lithium in 85 ml. of dry ether, in a 200-ml. three-necked flask equipped with a stirrer, a reflux condenser and a dropping funnel. After preparation of the organolithium compound in an atmosphere of nitrogen, 20.8 g. (0.20 mole) of cycloöctatetraene was added, the reflux condenser was replaced by a 20 × 1.5-cm. Vigreux column, and the mixture was gradually heated to 90° (bath temperature) during 45 minutes as the ether distilled. Stirring and heating at that temperature were continued for an additional period of 2 hours, after which the mixture was cooled in ice and 50 ml. of ether was added, followed by 100 ml. of water which was added slowly with cooling and stirring. The red ether layer was separated and combined with a 25-ml. ethereal extract of the aqueous layer. The solution was washed with water, and then was extracted with four 50-ml. portions of 4% hydrochloric acid to separate basic products. The ethereal solution was washed once with water, and the combined aqueous and acid extracts were made basic with 25% aqueous sodium hydroxide. The red liquid which separated was extracted with three 50-ml. portions of ether, and the combined extracts were washed three times with water, dried over magnesium sulfate, and concentrated. The red liquid residue was distilled through a semimicro column in order to separate 4.5 g. of N,N-dimethylaniline, b.p. 96–98° (32 mm.), from a high-boiling residue. The residue was distilled in a short-path still at 0.4 mm. with a heating block temperature of 200°. The red liquid distillate solidified rapidly, yielding crude *p*-dimethylaminophenylcycloöctatetraene (IV) (8.2 g., 37%) as an orange solid. The crude product was dissolved in the minimum amount (65 ml.) of hot methanol, in which it dissolved slowly, and the solution was seeded and allowed to cool slowly. IV crystallized as small orange leaflets, which were separated by filtration, pressed dry on the funnel, and recrystallized again in the same way. The yield of pure IV, m.p. 89.5–90.7°, was 5.9 g. (27%). An analytical sample was crystallized from ethanol to a constant melting point of 90–90.5°.

Anal. Calcd. for $C_{16}H_{17}N$: C, 86.05; H, 7.68; N, 6.27. Found: C, 86.34; H, 7.75; N, 6.27.

p-Dimethylaminophenylcycloöctatetraene picrate was prepared by adding a solution of 224 mg. of IV in 5 ml. of ether to a solution of 253 mg. of picric acid in 10 ml. of ether. The picrate precipitated as a yellow-green solid (412 mg., 91%), m.p. 126–127° (dec.). Recrystallization from 95% ethanol did not change the melting point. The picrate changed rapidly from yellow-green to deep green in color on standing.

Anal. Calcd. for $C_{22}H_{20}N_4O_7$: C, 58.40; H, 4.46; N, 12.39. Found: C, 58.26; H, 4.74; N, 12.41.

A solution of 0.418 g. of IV in 10 ml. of methanol absorbed 99% of four molar equivalents of hydrogen in two hours in the presence of 0.3 g. of 10% palladium-on-carbon catalyst.[15] After separation of the catalyst, distillation yielded 0.26 g. (61%) of *p*-dimethylaminophenylcycloöctane, b.p. 145–147° (0.3 mm.), n^{25}_{D} 1.5520. The picrate of *p*-dimethylaminophenylcycloöctane was prepared from the amine and picric acid in hot 95% ethanol, and recrystallized from the same solvent as yellow crystals, m.p. and mixed m.p. with an authentic sample described below, 149–150°.

Anal. Calcd. for $C_{22}H_{28}N_4O_7$: C, 57.38; H, 6.13; N, 12.17. Found: C, 57.20; H, 6.31; N, 12.32.

The ethereal solution containing products of the reaction of *p*-dimethylaminophenyllithium and cycloöctatetraene which were not extracted by 4% hydrochloric acid was concentrated, and the residue was distilled through a semimicro column,[14] yielding 14.4 g., b.p. 76–79° (92 mm.), n^{25}_{D} 1.5248. Extraction of a solution of this material in 25 ml. of pentane with seven 25-ml. portions of 20% silver nitrate by a procedure described above separated 8.7 g. (42%) of recovered cycloöctatetraene, b.p. 73–74° (88 mm.), n^{25}_{D} 1.5323, and 3.83 g. (36%) of a mixture of cycloöctatrienes, b.p. 78–80° (92 mm.), n^{25}_{D} 1.5122. Treatment of this mixture with potassium *t*-butoxide in *t*-butyl alcohol yielded 3.33 g. of 1,3,5-cycloöctatriene, b.p. 80–81° (90 mm.), n^{25}_{D} 1.5199, $\lambda_{max.}$ 266 mμ (ϵ 3450); $\lambda_{min.}$ 226 mμ (ϵ 1080), m.p. of the maleic anhydride adduct and mixed m.p. with a known sample,[9] 143.5–145°.

p-Dimethylaminophenylcycloöctene (V) was prepared by adding a solution of 13.0 g. of 3-bromocycloöctene[13] in 30 ml. of dry ether to the *p*-dimethylaminophenyllithium prepared from 12.0 g. of *p*-bromodimethylaniline and 0.92 g. of lithium in 100 ml. of dry ether. The addition required 10 minutes, and the mixture was stirred and heated under reflux for 1 hour. After cooling, 50 ml. of water was added, and the ethereal layer was separated, washed with water, and extracted with two 50-ml. portions of 4% hydrochloric acid. The acidic extracts were washed once with ether, then made basic with 25% sodium hydroxide, and the product was extracted with two 50-ml. portions of ether. The combined extracts were washed with water, dried over magnesium sulfate, concentrated, and the residue was distilled through a semi-micro column. V was obtained in a yield of 4.63 g. (34%) as an unstable liquid, b.p. 140–149° (0.4 mm.), n^{25}_{D} 1.5673. An analytical sample was obtained by redistillation, b.p. 148–149° (0.3 mm.), n^{25}_{D} 1.5662.

Anal. Calcd. for $C_{16}H_{23}N$: C, 83.79; H, 10.11; N, 6.10. Found: C, 83.97; H, 10.20; N, 6.18.

p-Dimethylaminophenylcycloöctene picrate was prepared from V and picric acid in hot 95% ethanol, and recrystallized from the same solvent as yellow crystals melting at 154–155° (dec.).

Anal. Calcd. for $C_{22}H_{26}N_4O_7$: C, 57.65; H, 5.72; N, 12.22. Found: C, 58.01; H, 5.87; N, 12.16.

p-Dimethylaminophenylcycloöctane (VI) was prepared by hydrogenation of a solution of 0.653 g. of V in 15 ml. of methanol in the presence of 0.9 g. of 10% palladium-on-carbon. The reduction required 6 hours for completion and 140% of one molar equivalent of hydrogen was absorbed. After separation of the catalyst, distillation yielded 0.35 g. of *p*-dimethylaminophenylcycloöctane (VI), b.p. 133–139° (0.1 mm.), n^{25}_{D} 1.5519.

Anal. Calcd. for $C_{16}H_{25}N$: C, 83.05; H, 10.89; N, 6.06. Found: C, 82.95; H, 11.03; N, 6.23.

An authentic sample of *p*-dimethylaminophenylcyclooctane picrate was obtained by treating VI prepared in this manner with picric acid in 95% ethanol. On recrystallization from 95% ethanol the picrate formed yellow leaflets melting at 149–150°.

p-Dimethylaminophenylcycloöctatetraene *d*-Camphor-10-sulfonate.—*d*-Camphor-10-sulfonic acid, 0.46 g., was added to a solution of 0.48 g. of IV in 30 ml. of U.S.P. ether containing 0.5 ml. of water. The mixture was warmed and 1.7 ml. of 95% ethanol was added dropwise until the oil which had formed dissolved. The yellow solution was cooled slightly, seeded with a crystal of the salt obtained from an earlier preparation, and gradually cooled to −80° with stirring. The salt was collected on a filter and air-dried; it amounted to 0.73 g. of pale yellow leaflets of a dihydrate, m.p. 56–57°, $[\alpha]^{28}_{D}$ +24.4° (l = 1, c = 3.82 in absolute ethanol).

Anal. Calcd. for $C_{26}H_{33}NO_4S{\cdot}2H_2O$: C, 63.51; H, 7.59; H_2O, 7.33. Found: C, 63.28; H, 7.61; H_2O, 7.06 (loss in weight on drying to constant weight during a period of 4 hours at 25° and 25 mm.).

Anhydrous *p*-dimethylaminophenylcycloöctatetraene *d*-camphor-10-sulfonate was obtained by drying the dihydrate at 0.5 mm. and 25°; m.p. 111–112.5° (dec.).

Anal. Calcd. for $C_{26}H_{33}NO_4S$: C, 68.54; H, 7.31; N, 3.07; S, 7.04. Found: C, 68.30; H, 7.37; N, 3.32; S, 6.89.

A sample of the *d*-camphor-10-sulfonate dihydrate was recrystallized three times by suspending it in boiling U.S.P. ether, adding 95% ethanol until the solution became clear, and cooling to −80° with stirring after seeding. The salt was unchanged in melting point and rotation, within experimental error; m.p. 56–57°, $[\alpha]^{28}_{D}$ +24.2° (l = 1, c = 3.35 in absolute ethanol). A solution of 110 mg. of the recrystallized salt in 3 ml. of chloroform was washed with eight 5-ml. portions of water, which served to hydrolyze the salt and remove the *d*-camphor-10-sulfonic acid. The chloroform solution had a rotation of zero, and on evaporation and crystallization of the residue from 1 ml. of methanol yielded 30 mg. of IV, m.p. 90–91°.

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(18) H. Gilman, E. A. Zoellner and W. M. Selby, THIS JOURNAL, **55**, 1252 (1933).

Reaction of Phenylmagnesium Bromide with Cycloöctatetraene.—A solution of phenylmagnesium bromide was prepared from 31.4 g. (0.20 mole) of bromobenzene and 4.86 g. of magnesium in 90 ml. of dry ether. Cycloöctatetraene (20.8 g., 0.20 mole) was added, and the homogeneous solution that was formed was stirred and heated to a bath temperature of 100°. After 1 hour the brown liquid mixture had changed to a bright yellow solid; stirring was discontinued, and heating at 100° was continued for 1 hour longer. The mixture was cooled with ice, and 125 ml. of a cold, saturated solution of ammonium chloride in water was added with stirring. The yellow ethereal layer was separated, and the aqueous layer was extracted with 50 ml. of ether. The combined ethereal solutions were washed with water, dried over magnesium sulfate, and concentrated. Two fractionations of the low boiling material through a semi-micro column separated fraction 1, 16.3 g., b.p. 73–78° (89 mm.), $n^{25}D$ 1.5268. This fraction was determined to contain 2.4% of bromobenzene by quantitative analysis for bromine, 71% (56% recovery) of cycloöctatetraene by colorimetric analysis,[16] and 27% or 4.4 g. of cyclooctatrienes (42% yield) by difference. Extraction with 20% silver nitrate by the procedure previously described separated 8.44 g. (41% recovery) of cycloöctatetraene, b.p. 74–75° (91 mm.), $n^{25}D$ 1.5322, and 3.33 g. (31%) of cyclooctatrienes, b.p. 78–80° (90 mm.), $n^{25}D$ 1.5132. Treatment with potassium *t*-butoxide in *t*-butyl alcohol yielded 2.18 g. of crude 1,3,5-cycloöctatriene, b.p. 80–82° (92 mm.), $n^{25}D$ 1.5222, which was identified by conversion to the maleic anhydride adduct, m.p. 143–144°.

The high boiling residue from which fraction 1 was separated crystallized on cooling. It was recrystallized from ethanol and sublimed at 0.5 mm. with a heating block temperature of 75–80°, and yielded 5.8 g. of biphenyl, m.p. 69.5–70.5°. An additional 1.2 g. was obtained from the ethanol mother liquor by sublimation, making the total yield 7.0 g. (45%). The residue from the sublimation was a dark orange resin.

A control experiment was conducted to determine what proportion of the biphenyl isolated from the reaction of phenylmagnesium bromide with cycloöctatetraene could be derived from the Grignard reagent without participation of cycloöctatetraene in the reaction. A solution of phenylmagnesium bromide was prepared from 31.4 g. of bromobenzene and 4.86 g. of magnesium in 90 ml. of ether. Ethylcyclohexane (20.8 g.) was added and the mixture (two layers) was stirred and heated to a bath temperature of 100°, while 30 ml. of ether distilled. Stirring and heating at 100° were continued for 2 hours, after which the mixture was cooled, hydrolyzed, and the product was isolated in the same manner as the products obtained from phenylmagnesium bromide and cycloöctatetraene. The yield of biphenyl, m.p. 68–70°, was 1.02 g. (6.6%).

CAMBRIDGE, MASS. RECEIVED OCTOBER 30, 1950

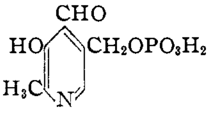
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Phosphates of the Vitamin B_6 Group. I. The Structure of Codecarboxylase

BY DOROTHEA HEYL, EILEEN LUZ, STANTON A. HARRIS AND KARL FOLKERS

Evidence which is presented indicates that codecarboxylase is the monophosphoric acid ester of the 5-hydroxymethyl group of pyridoxal. It has been isolated in pure form as the oxime and as the O-methyloxime. The oxime of the betaine of 1,2-dimethyl-3-hydroxy-4-formyl-5-pyridylmethylphosphoric acid has also been obtained. The calcium salt of 2-methyl-3-hydroxy-4-hydroxymethyl-5-pyridylmethylphosphoric acid has been obtained after phosphorylation of pyridoxine with phosphorus oxychloride in the presence of water.

Codecarboxylase has been synthesized, and evidence is now available to support the conclusion that it is the monophosphoric acid ester, I, of the 5-hydroxymethyl group of pyridoxal.



I

Codecarboxylase[1] was found to be a phosphorylated derivative of pyridoxal; later its activity as a cotransaminase[2] in the synthesis of amino acids was discovered. Pyridoxal phosphate was originally prepared by the action of adenosine triphosphate on pyridoxal.[1] It was prepared later by the action of phosphorus oxychloride on pyridoxal in the presence of water.[3]

Codecarboxylase is formed in low yield by phosphorylation of pyridoxal in aqueous solution with phosphorus oxychloride, and has been isolated as a calcium salt by direct neutralization of the phosphorylation mixture with calcium carbonate. The calcium phosphate is precipitated, leaving codecarboxylase in solution. The addition of ethyl alcohol to the solution precipitates the calcium salt of the coenzyme. This material has been estimated by assay to contain 50–80% of codecarboxylase.[4]

Codecarboxylase has been characterized as a pure crystalline oxime,[5] for which the formula $C_8H_{11}N_2O_6P$ was demonstrated. The same oxime was prepared by phosphorylation of pyridoxal oxime with phosphorus oxychloride in aqueous solution. The formula for the oxime also established the formula $C_8H_{10}NO_6P$ for codecarboxylase itself, showing it to be a condensation product of pyridoxal and phosphoric acid with the elimination of one molecule of water. The analytical data preclude the presence of a phosphoric acid ester group on either the 6-position or the 2-methyl group.

Positive ferric chloride color tests for both the calcium salt and the oxime of codecarboxylase show the presence of a free phenolic hydroxyl group. This result was substantiated by the synthesis of 3-pyridoxalphosphoric acid oxime, which was not identical with codecarboxylase oxime.[5,6] The formyl group (in hemi-acetal linkage with the 5-hydroxymethyl group) was eliminated as the location of the phosphate group both by the formation of the oxime without elimination of the phosphate

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[1] Gunsalus, Bellamy and Umbreit, *J. Biol. Chem.*, **155**, 685 (1944).

[2] Lichstein, Gunsalus and Umbreit, *ibid.*, **161**, 311 (1945).

[3] Gunsalus, Umbreit, Bellamy and Faust, *ibid.*, **161**, 743 (1945).

[4] Umbreit, Bellamy and Gunsalus, *Arch. Biochem.*, **7**, 185 (1945). We are indebted to Drs. Gunsalus and Umbreit for assaying our preparations.

[5] Heyl, Harris and Folkers, Abstracts, American Chemical Society, 110th Meeting, Chicago, 35B (1946).

[6] Heyl and Harris, THIS JOURNAL, **73**, 3434 (1951).