Cl, 7.41; Hg, 41.88; O, 3.34; S, 6.68. Found: C, 37.21; H, 3.27; Cl, 7.01; Hg, 41.50; O, 3.14; S, 6.32.

The residue from the ether solution was chromatographed on 20 g. of Merck acid-washed alumina and yielded 110 mg. of recovered hemithioketal XII and 235 mg. of cholestan-3-one, m.p. and mixture m.p. 128–129°.

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STANFORD UNIVERSITY]

The Catalytic Hydrogenation of 3-Phenyl-1-butene-2-C^{14}

BY WILLIAM A. BONNER, CHARLES E. STEHR AND JEFFERSON R. DO AMARAL[1]

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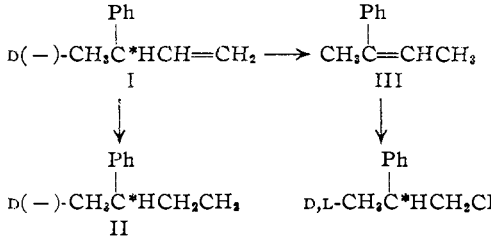
D(−)-3-Phenyl-1-butene undergoes varying extents of racemization on catalytic hydrogenation to D(−)-2-phenylbutane.[2,3] A rationalization[3] of this fact, involving double bond migration to 2-phenyl-2-butene prior to reduction, appears[4] untenable. An alternative rationalization, involving partial hydrogenation to a 3-phenyl-2-butyl radical followed by non-stereospecific phenyl migration, has been tested by hydrogenation of uniquely labeled 3-phenyl-1-butene-2-C^{14} (IV) under a variety of conditions. The resulting labeled 2-phenylbutane samples have been oxidized to benzoic acid samples, radioactivity assays of which indicate the extents of net phenyl migration attending each catalytic hydrogenation. The net phenyl migrations observed were uniformly less than one per cent., eliminating both the above rationalization and a bridged radical intermediate as important mechanisms for racemization. An alternative mechanism involving hydrogen migration is suggested.

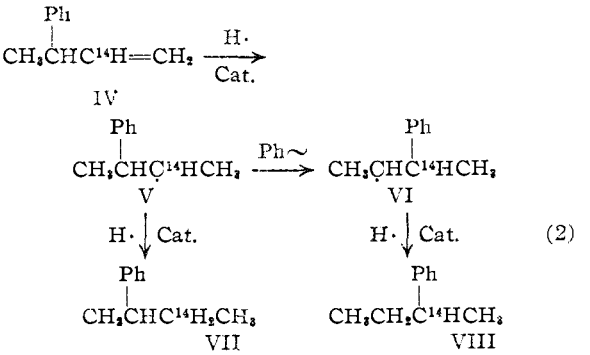
In 1952, Cram observed[2] that when D(−)-3-phenyl-1-butene (I) was catalytically hydrogenated under a variety of conditions the resulting D(−)-2-phenylbutane[3] (II) was racemized to the extent of 1 to 11%. Since D(−)-2-phenylbutane itself was optically stable under the experimental conditions of the catalytic hydrogenation, Cram concluded[2] that the racemization must have occurred prior to or during the hydrogenation process, and suggested that the olefin I might have rearranged partially into conjugated 2-phenyl-2-butene (III) prior to hydrogenation (equation 1), subsequent reduction



(1)

of III producing the racemic 2-phenylbutane. More recently Huntsman and Schlesinger, in a study of double bond migration and racemization during the hydrogenation of olefins, have similarly observed[4] extensive racemization (*ca.* 60%) during the hydrogenation of optically active 3-phenyl-1-butene in the presence of palladium–charcoal catalyst. They have reported further that double bond migration is much slower than hydrogenation in this case and that racemization failed to occur in the absence of hydrogen. They concluded, therefore, that racemization of optically active 3-phenyl-1-butene during hydrogenation must occur by some mechanism other than double bond migration to the asymmetric center.

An alternative mechanism to double bond migration which would account for the racemization of 3-phenyl-1-butene during hydrogenation occurred to us in 1954 and will now be described. If a portion of the starting olefin underwent *partial, stepwise* reduction, rather than instantaneous complete reduction by addition of hydrogen atom pairs,[5] an intermediate secondary free radical such



(2)

as V could result. If V were sufficiently long-lived, phenyl migration might occur yielding the equivalent rearranged radical VI, with its radical site at the previous asymmetric center. Lacking any marked stereospecificity in the phenyl migration step or in the subsequent further reduction of rearranged radical VI, the final 2-phenylbutane from such a process should be substantially racemic. Reduction of the unrearranged radical V would, of course, yield unracemized 2-phenylbutane. A racemization mechanism such as (2) is a reasonable one in view both of the demonstrated[6–11] tendencies of the phenyl group to undergo migration in reactions known to involve free radical intermediates and of the recent report[12] that rearrangement accompanies the palladium–charcoal catalyzed reduction of certain halogenated bicyclo[2,2,1]heptane compounds.

The intervention of a mechanism such as (2) to engender racemization during the hydrogenation

(1) We are indebted to the National Science Foundation for its support of a portion of this investigation.

(2) D. J. Cram, THIS JOURNAL, **74**, 5518 (1952).

(3) D. J. Cram, *ibid.*, **74**, 2150 (1952).

(4) W. D. Huntsman and S. I. Schlesinger, Abstract in "A Report on Research under Sponsorship of the Petroleum Research Fund Administered by the American Chemical Society," 1954–1956, p. 18.

(5) R. P. Linstead and co-workers, THIS JOURNAL, **64**, 1983 (1942).

(6) W. H. Urry and M. S. Kharasch, *ibid.*, **66**, 1438 (1944).

(7) S. Winstein and F. H. Seubold, *ibid.*, **69**, 2916 (1947).

(8) D. Y. Curtin and M. S. Hurwitz, *ibid.*, **74**, 5381 (1952).

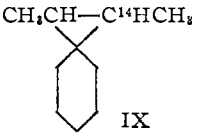
(9) S. Winstein, *Experientia*, **12**, 138 (1956).

(10) D. F. DeTar and A. Hlynsky, THIS JOURNAL, **77**, 4411 (1955).

(11) E. Grovenstein, Jr., *ibid.*, **79**, 4985 (1957).

(12) H. Kwart and G. Null, *ibid.*, **80**, 248 (1958).

of 3-phenyl-1-butene could be demonstrated readily by employing an appropriately labeled olefin starting material, namely, 3-phenyl-1-butene-2-C^{14} (IV). Any rearrangement such as postulated in (2) would then involve phenyl migration to the labeled site of the radical intermediate. Such migration could be estimated both qualitatively and quantitatively by oxidation of the labeled 2-phenylbutane product (VIII and/or VII) to benzoic acid, comparative radioactivity assays of the final benzoic acid and the olefin IV giving a precise indication of the net phenyl migration occurring during the hydrogenation. Additionally, such an experiment might shed light on the possibility of a bridged phenyl radical IX, a non-



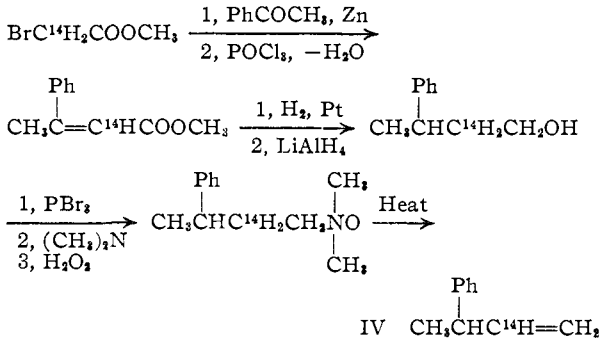
IX

classical intermediate analogous to the well established "symmetrical phenonium ion" cationic intermediate.[13] Experimental indications of bridged radical intermediates have recently been sought in vain in a number of other free radical reactions.[14]

The 3-phenyl-1-butene-2-C^{14} (IV) required to test experimentally the hypothesis of mechanism 2 was synthesized by the sequence of reactions indicated in Chart I, starting with commercially available methyl bromoacetate-2-C^{14}. The reaction sequence beyond the 3-phenyl-1-butanol-2-C^{14} stage is similar to that employed by Cram[15] for the synthesis of D(−)-3-phenyl-1-butene, uncontaminated by any conjugated 2-phenyl-2-butene. The location of the labeled carbon atom in IV uniquely at the 2-position follows from the preparative scheme in Chart I. To establish this

CHART I

SYNTHESIS OF 3-PHENYL-1-BUTENE-2-C^{14}

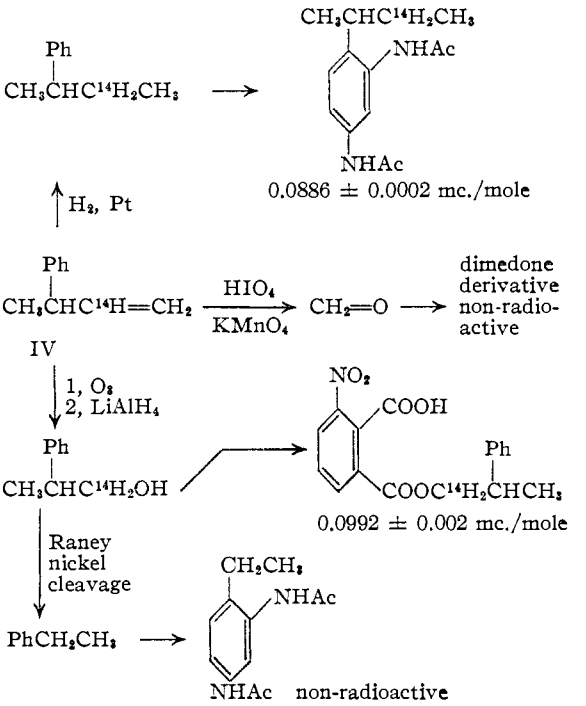


location experimentally, however, seemed additionally desirable. The degradative scheme constituting the radiochemical structure proof of IV is indicated in Chart II.

3-Phenyl-1-butene-2-C^{14} was catalytically hydrogenated and the labeled 2-phenylbutane product

CHART II

RADIOCHEMICAL STRUCTURE PROOF OF 3-PHENYL-1-BUTENE-2-C^{14}



converted to its solid 2,4-diacetylamino derivative, assay of which gave the radioactivity level of the entire molecule IV. Carbon-1 of olefin IV was examined separately by oxidation of IV with potassium permanganate and periodic acid after the method of Lemieux and von Rudloff,[16] followed by conversion of the resulting formaldehyde to its dimedone derivative. The latter proved to be devoid of radioactivity. Olefin IV was next subjected to ozonization followed by reduction of the ozonide with lithium aluminum hydride. The resulting labeled 2-phenyl-1-propanol yielded a solid acid 3-nitrophthalate derivative of radioactivity assay in substantial agreement with that characteristic of IV. Carbon atoms 2 and 3 of the labeled 2-phenyl-1-propanol were examined jointly by cleavage of this alcohol with Raney nickel[17] under conditions known[18] to involve no rearrangement. The resulting ethylbenzene was converted to its solid 2,4-diacetylamino derivative, which proved to be non-radioactive. These observations uniquely establish the radiochemical structure of our 3-phenyl-1-butene-2-C^{14}.

The olefin IV was next catalytically reduced to labeled 2-phenylbutane at atmospheric hydrogen pressure with the catalysts indicated in Table I, conditions known to produce varying amounts of racemization with D(−)-3-phenyl-1-butene.[2,4] The resulting 2-phenylbutane samples were oxidized by a two-step procedure to benzoic acid samples which were purified and assayed for radioactivity. Division of each benzoic acid radioactivity assay by

(13) D. J. Cram and co-workers, THIS JOURNAL, **71**, 3863, 3871, 3875 (1949); **74**, 2129, 2137, 2159 (1952); **75**, 3189 (1953); S. Winstein and co-workers, *ibid.*, **61**, 1576 (1939); **77**, 4183 (1955).

(14) *Cf.* S. J. Cristol, G. D. Brindell and J. A. Reeder, *ibid.*, **80**, 635 (1958), for references to failures to detect non-classical free radical intermediates.

(15) D. J. Cram, *ibid.*, **74**, 2146 (1952).

(16) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1701 (1955).

(17) J. A. Zderic, W. A. Bonner and T. W. Greenlee, THIS JOURNAL, **79**, 1696 (1957).

(18) W. A. Bonner and T. W. Greenlee, unpublished observations to be disclosed in detail in a future communication.

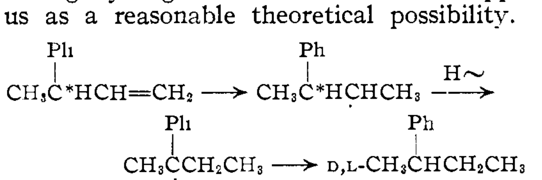
the "base level" olefin IV assay (0.0939±0.0053 mc./mole) gives the fraction of net phenyl migration occurring during each hydrogenation of IV under the conditions summarized in Table I. All hydrogenations were conducted for a three-hour period except no. 4, which was allowed to proceed for one week in order to test the behavior of the labeled 2-phenylbutane product under such hydrogenation conditions.

TABLE I

NET PHENYL MIGRATION DURING CATALYTIC HYDROGENATION OF 3-PHENYL-1-BUTENE-2-C^{14}

Hydrogenation no.	Catalyst	Benzoic acid assay, (mc./mole) × 10^4	Net phenyl migration, %
1	PtO_2	5.54	0.59
2	Raney nickel	0.045	.0048
3	Pd–$CaCO_3$	6.54	.70
4	Pd–$CaCO_3$	8.42	.90
5	Pd–C	7.40	.95

Table I indicates that *less than one per cent.* net phenyl migration occurred under any of the hydrogenation conditions employed to reduce 3-phenyl-1-butene-2-C^{14}. It is interesting to note that the greatest net phenyl migrations were observed with those catalysts which led to the largest amounts of racemization[2,4] during hydrogenation of D(−)-3-phenyl-1-butene, but the differences in the net phenyl migration figures are too small to be considered particularly significant. The almost negligible extents of phenyl migration attending the hydrogenations of 3-phenyl-1-butene-2-C^{14} indicate clearly that mechanism 2 is of no importance in engendering racemization during the hydrogenation of optically active 3-phenyl-1-butene, and further demonstrate that a non-classical radical intermediate such as IX is involved at best only trivially in the present system. Since mechanism 2 is unimportant in racemizing optically active 3-phenyl-1-butene on hydrogenation, since 2-phenylbutane is optically stable under the reducing conditions employed[2] and since reduction of 3-phenyl-1-butene is much faster than double bond migration to an intermediate optically inactive 2-phenyl-2-butene,[4] a fourth alternative must be sought to explain the racemization of 3-phenyl-1-butene during hydrogenation. Mechanism 3 appears to us as a reasonable theoretical possibility. It is



(3)

similar to our experimentally investigated mechanism 2, except that hydrogen instead of phenyl is the migrating species. While we are aware of no demonstrated case of hydrogen migration in a free radical intermediate, a process such as (3) might be quite feasible on a catalyst surface containing adsorbed hydrogen. Furthermore, mechanism 3 might receive its driving force in the conversion of the unstabilized secondary radical intermediate to the resonance-stabilized tertiary radical successor, which would presumably lose much if not all of its optical integrity. An experimental investigation of mechanism 3 employing deuterium-labeled analogs is in progress.

Experimental

Methyl β-methylcinnamate-α-C^{14} was prepared by the Reformatsky reaction of acetophenone (53.3 g.) with methyl bromoacetate-2-C^{14} (52 g.) according to the procedure of Lipkin and Stewart.[19] The crude product was fractionated, and the fraction having b.p. 136–138° (18 mm.), 30.1 g. (50.3%), was collected.

Methyl β-Phenylbutanoate-2-C^{14}.—The above labeled cinnamic ester (32.6 g.) was dissolved in absolute ethanol (26 ml.), treated with platinum oxide catalyst (1.3 g.) and subjected to hydrogenation in a Parr low pressure hydrogenator. The theoretical pressure drop of hydrogen was observed. After filtration and solvent removal the crude methyl 3-phenylbutanoate-2-C^{14} weighed 32.5 g. (98.6%) and showed $n^{19.5}D$ 1.4972.

3-Phenyl-1-butanol-2-C^{14}.—The above methyl 3-phenylbutanoate-2-C^{14} (32.5 g.) in ether solution was reduced to 3-phenyl-1-butanol-2-C^{14} using lithium aluminum hydride (3.9 g.) after the procedure of Nystrom and Brown.[20] The crude product, 27 g. (97.6%), was used below without purification.

3-Phenyl-1-butyl-2-C^{14} Bromide.—The above carbinol (27 g.) was converted to the corresponding bromide by means of phosphorus tribromide, following the procedure of Rupe and van Walvaren.[21] The crude product was distilled *in vacuo*, yielding 31.2 g. (81%) of pure material having b.p. 123–124° (19 mm.) and $n^{21.5}D$ 1.5341.

Dimethyl-(3-phenyl-1-butyl-2-C^{14})-amine.—A mixture of the above 3-phenyl-1-butyl-2-C^{14} bromide (33.2 g.), dimethylamine (38 g.) and purified dioxane (64 ml.) was chilled and sealed into a Pyrex bomb tube. The tube was heated at 100° for 12.5 hours, cooled and opened. The resulting tertiary amine was isolated and freed of contaminants by the procedure of Cram.[15] There was isolated 26.7 g. (96.7%) of crude amine which was used directly in the oxidation below.

Dimethyl-(3-phenyl-1-butyl-2-C^{14})-amine Oxide.—The above tertiary amine (26.7 g.) was converted to its amine oxide by oxidation with 30% hydrogen peroxide (40 g.) after the method of Cram.[15] The excess oxidant was destroyed after 24 hours by means of the catalase in an aqueous extract of avocado skins, and the crude amine oxide was concentrated by lyophilization.[22] The crude residue was used directly in the pyrolysis below.

3-Phenyl-1-butene-2-C^{14} (IV).—The general procedure of Cram[15] was employed for the pyrolytic conversion of the above amine oxide into 3-phenyl-1-butene-2-C^{14}. The pyrolysis was conducted in a Claisen flask under a 5 mm. nitrogen atmosphere, heating the flask gradually from 90 to 140° and collecting the crude olefin product in a Dry Ice trap. The condensate was dissolved in pentane and the solution was washed with dilute hydrochloric acid, then dilute sodium hydroxide solution. After drying, the solvent was distilled off through a column, and the residue fractionally distilled. An 8.7-g. (42%) fraction having b.p. 66–68° (19 mm.) and $n^{23.5}D$ 1.5043 was collected, whose infrared spectrum was identical to that of a stock sample and to that reported by Cram,[15] and whose purity wss further confirmed by examination with the aid of a vapor–liquid partition chromatographic column, whereupon only a single recorded peak was observed.

Conversion of 3-Phenyl-1-butene-2-C^{14} to Formaldehyde.—In order to estimate the label content at carbon-1 of IV, an olefin, degradation of the olefin to formaldehyde (arising from carbon-1) was undertaken, adapting the procedure of Lemieux and von Rudloff.[16] A solution of paraperiodic acid (4 g.) in water (400 ml.) was neutralized with sodium carbonate, then treated with 0.5 g. of the 3-phenyl-1-butene-2-C^{14}. The mixture was stirred vigorously (Vibromixer) and treated with a solution of potassium permanganate (0.33 g.) in water (25 ml.) in four equal portions over a period of one hour. The mixture was stirred for an additional two hours, the excess permanganate was destroyed by addition of suffi-

(19) D. Lipkin and T. D. Stewart, THIS JOURNAL, **61**, 3295 (1939).

(20) R. F. Nystrom and W. G. Brown, *ibid.*, **69**, 2548 (1947).

(21) H. Rupe and F. van Walvaren, *Helv. Chim. Acta*, **13**, 369 (1930).

(22) D. H. Campbell and D. Pressman, *Science*, **99**, 285 (1944).

cient sodium bisulfite, and the oxidation mixture was extracted four times with ether. The aqueous layer was filtered through Celite and the resulting clear solution was treated with an aqueous solution containing a slight excess of dimedone reagent. The formaldehyde dimedone derivative precipitated instantly and was collected; 0.47 g. (40.3%), m.p. 187–187.5°. The derivative was recrystallized once from 60% ethanol prior to its radioactivity assay, m.p. 189° in agreement with the literature.[23] The product was devoid of radioactivity.

Ozonization of 3-Phenyl-1-butene-2-C^{14}.—A solution of IV, 3-phenyl-1-butene-2-C^{14}, (0.56 g.) in anhydrous ether (20 ml.) was surrounded by a cooling bath (6–8°) and ozonized during 55 minutes in a semi-micro ozonizer,[24] whereupon the ozonization mixture was added gradually with stirring to a suspension of lithium aluminum hydride (0.4 g.) in ether (30 ml.). The excess hydride was decomposed by the cautious addition of sufficient 10% sulfuric acid, and the ether layer was washed and dried over anhydrous sodium sulfate. Solvent removal left 0.56 g. (97.2%) of 2-phenyl-1-propanol-1-C^{14}, whose infrared spectrum was identical with that of a stock sample and which showed only a single peak when analyzed on a vapor–liquid partition chromatographic column. This ozonization and reduction procedure was repeated several times with comparable results.

2-Phenyl-1-propyl-1-C^{14} Hydrogen 3-Nitrophthalate.—A mixture of the 2-phenyl-1-propanol-1-C^{14} (0.56 g.), freshly recrystallized 3-nitrophthalic anhydride (0.7 g.) and anhydrous toluene (4 ml.) was heated under reflux for 20 minutes, then allowed to cool slowly. The first crop of crystals, 0.33 g. (25%), m.p. 136–139°, was collected. It was recrystallized from toluene four times to a constant m.p. 143–143.5° prior to radioactivity assay.

Anal. Calcd. for $C_{17}H_{15}O_6N$: C, 62.00; H, 4.59. Found: C, 61.93, 61.78; H, 4.57, 4.70; specific radioactivity, 0.0994, 0.0990 mc./mole.

Cleavage of 2-Phenyl-1-propanol-1-C^{14} with Raney Nickel.—To estimate the radioactivity content of carbons 2 and 3 in 2-phenyl-1-propanol-1-C^{14}, the compound was cleaved to ethylbenzene by the action of Raney nickel in refluxing ethanol.[17] A mixture of 2-phenyl-1-propanol-1-C^{14} (0.76 g.), absolute ethanol (10 ml.) and Raney nickel[25] (*ca.* 4 g.) was heated under reflux during a period of six hours, then cooled and filtered free of catalyst, rinsing the latter twice with ethanol (5 ml.). The filtrate was diluted with water (140 ml.) and the aqueous solution was extracted five times with pentane. The pentane extract was washed with water, dried over phosphoric anhydride and filtered, whereupon the solvent was removed by distillation through a fractionating column. The crude residue, containing[17] approximately 9 parts of ethylbenzene to 1 part of cumene, weighed 0.4 g. The ethylbenzene component was obtained pure by separation of the crude product on a 0.5 × 48 inch vapor–liquid partition chromatographic column[17] operated at a column temperature of 125° and using helium at 1.5 p.s.i. as carrier gas. The column packing consisted of crushed, acid-washed firebrick (6 parts) impregnated with high-boiling silicone oil (4 parts). The ethylbenzene fraction (0.12 g.) was recovered by chilling the exit gas stream during the interval when the ethylbenzene peak was being recorded.[17]

The ethylbenzene fraction was treated with a nitrating mixture containing sulfuric (2 ml.) and nitric (1 ml.) acids after the procedure of Ipatieff and Schmerling.[26] Reduction of the resulting dinitro and acetylation of the diamino derivative followed the published procedure.[26,27] The resulting crude 2,4-diacetylaminoethylbenzene, 0.052 g. (21%), was recrystallized from dilute ethanol and subjected to vacuum sublimation prior to radioactivity assay. The melting point of the purified product was 224°, in agreement with the literature value.[26,27] The purified product was non-radioactive.

Catalytic Hydrogenation of 3-Phenyl-1-butene-2-C^{14}.—Five separate catalytic hydrogenation experiments were undertaken on 1.00-g. samples of the above 3-phenyl-1-butene-2-C^{14} dissolved in 15 ml. of absolute ethanol, using an atmospheric pressure buret hydrogenator and stirring the hydrogenation mixture with a magnetic stirrer. The catalysts, hydrogen uptake and 2-phenylbutane yields are recorded in Table II. All of the hydrogenations were conducted for a 3-hour period except no. 4, which was conducted for a one-week period to test the effect of prolonged exposure to hydrogen and catalyst on possible phenyl migration. The typical procedure for isolation of the labeled 2-phenylbutane product follows. The hydrogenation mixture was filtered free of catalyst, which was rinsed twice with 5-ml. portions of ethanol. The filtrates were added to water (75 ml.), and the mixture was extracted four times with pentane. The pentane extract was washed with water, dried over calcium chloride, filtered and freed of solvent through a fractionating column. The crude residue showed $n^{22.5}D$ 1.4878, had an infrared spectrum in essential agreement with that of stock 2-phenylbutane, and showed no contaminant other than a trace of residual solvent when analyzed on the above vapor–liquid partition chromatographic column, the efflux time agreeing with that of stock 2-phenylbutane.

TABLE II

CATALYTIC HYDROGENATIONS OF 3-PHENYL-1-BUTENE-2-C^{14}

Hydrogenation no.	Catalyst	Catalyst wt., g.	Hydrogen uptake, %	2-Phenylbutane yield g.	2-Phenylbutane yield %
1	PtO_2	0.2	100.5	0.89	88.7
2	Raney nickel	0.3	98.8	.84	83.6
3	1.2% Pd-$CaCO_3$	1.0	99.5	.78	77.6
4	1.2% Pd-$CaCO_3$	1.0	...	.83	82.6
5	10% Pd-C	1.0	100.0	.84	83.6

Oxidation of Labeled 2-Phenylbutane Samples to Benzoic Acid.—After considerable preliminary investigation we found that the most satisfactory method for the conversion of the labeled 2-phenylbutane samples to benzoic acid involved a two-stage oxidation. The hydrocarbon was first oxidized to acetophenone using chromium trioxide in acetic acid after the general procedure of Meyer and Bernhauer,[28] and the ketone then was oxidized to benzoic acid by means of sodium hypochlorite.[29] A description of typical procedure and results is given. A mixture of acetic acid (30 ml.) and acetic anhydride (5 ml.) was placed in a 100-ml. 3-neck flask equipped with stirrer and thermometer and surrounded by a salt–ice-bath. Sulfuric acid (5 ml.) was added at such a rate as to maintain the temperature below 15°. The mixture was cooled to 10° and chromium trioxide (4 g.) was added. A dropping funnel was attached, and a solution of 0.84 g. of labeled 2-phenylbutane from hydrogenation 2 in Table II dissolved in acetic acid (10 ml.) was added slowly with stirring, at such a rate that the temperature remained from 10–13°. The mixture was stirred for an additional two hours, then allowed to stand at room temperature for two days, poured into excess ice-water, neutralized with sodium carbonate and extracted three times with ether. The ether extract was washed, dried over calcium chloride, filtered and freed of solvent through a fractionating column. The yield of crude residual acetophenone was 0.72 g. (90%). Vapor–liquid partition chromatographic investigation of the crude product indicated it to be substantially pure acetophenone. The crude acetophenone samples were oxidized to benzoic acid by the following typical procedure, appropriately scaled to the quantity at hand. A mixture of acetophenone (1 g.), methanol (5 ml.) and 20% sodium hydroxide solution (10 ml.) was stirred rapidly while chlorine was bubbled in. As the temperature rose, additional sodium hydroxide solution (10 ml.) was added gradually. When a white precipitate (NaCl) became evident the chlorine addition was stopped and the mixture was stirred an additional 15 minutes, then treated with acetone to destroy excess hypochlorite, acidified with hydrochloric acid, cooled and extracted with four 25-ml. portions of ether. The ether solution was extracted thrice with 2 *N* sodium hydroxide solution, and the basic extract was acidified and extracted four times with ether.

(23) A. I. Vogel, "A Textbook of Practical Organic Chemistry," Longmans, Green and Co., London, 1954, p. 334.

(24) W. A. Bonner, *J. Chem. Educ.*, **30**, 452 (1953).

(25) R. Mozingo, *Org. Syntheses*, **21**, 15 (1941).

(26) V. N. Ipatieff and L. Schmerling, THIS JOURNAL, **59**, 1056 (1937).

(27) N. D. Cheronis, "Micro and Semimicro Methods," Interscience Publishers, Inc., New York, N. Y., 1954, p. 560.

(28) H. Meyer and K. Bernhauer, *Monatsh.*, **53**, 728 (1929).

(29) A. M. Van Arendonk and M. E. Cupery, THIS JOURNAL, **53**, 3184 (1931).

The latter ether extract was washed with water, dried over calcium chloride, filtered and distilled to dryness through a column. The residual crude benzoic acid, 0.89 g. (88%), was recrystallized twice from water, then vacuum sublimed prior to radioactivity assay, m.p. 122–122.5°. The radioactivity assays of the benzoic acid samples arising from each of the 2-phenylbutane samples of Table II are given in Table I.

2-(2,4-Diacetylaminophenyl)-butane-3-C^{14}.—3-Phenyl-1-butene-2-C^{14} (2.30 g.) was hydrogenated with the use of platinum oxide catalyst and hydrogen as described, yielding 1.91 g. (82%) of labeled 2-phenylbutane. This was nitrated at room temperature using 20 ml. of a 2:1 sulfuric–nitric acid mixture. The dinitro product was isolated and reduced, and the resulting diamino product was acetylated as described above for the preparation of the corresponding derivative of ethylbenzene. The crude 2-(2,4-diacetylaminophenyl)-butane-3-C^{14}, 0.79 g. (22%), m.p. 183–185°, was recrystallized thrice from 80% ethanol, when it had a constant m.p. of 189–190°, unchanged after vacuum sublimation. The m.p. of this derivative is given as 191.5°[30] and 193°[26]; radioactivity assay: 0.0888, 0.0884 mc./mole.

(30) T. E. Zalesskaya, *J. Gen. Chem. U.S.S.R.*, **17**, 489 (1947); *C. A.*, **42**, 844 (1948).

Radioactivity assays were accomplished by wet combustion of the labeled samples to carbon dioxide,[31] followed by counting[32] the latter in an ionization chamber with the aid of a Cary model 31 vibrating reed electrometer. Due to the extremely low levels of radioactivity in several of the samples assayed it was necessary to make corrections for background radiation and electrometer drift in order to minimize errors from these sources. In general this was accomplished by (a) correcting the slow observed rate of drift curves for "instantaneous plateaus" due to bursts of background radiation and (b) correcting the slow ion-current rate of drift for the slower rate of drift due to ion chamber charge leakage, the latter itself separately corrected for "instantaneous plateaus" caused by background radiations. Such corrections have been applied in the calculations of all of the radioactivity assays in Table I. The assays of the benzoic acid samples in Table I, while significantly above background level, are nevertheless so low that we are unable to estimate their absolute experimental validity at the present time.

(31) O. K. Neville, THIS JOURNAL, **70**, 3501 (1948).

(32) V. A. Raaen and G. A. Ropp, *Anal. Chem.*, **25**, 174 (1953).

STANFORD, CALIF.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE JOHNS HOPKINS UNIVERSITY]

Properties of Proto- and Mesoheme Imidazole Complexes[1]

BY ALSOPH H. CORWIN AND STEPHEN D. BRUCK

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An improved method for the preparation of proto- and mesohemes is presented. In the crystalline state, both imidazole proto- and mesohemochromes have the ability to combine reversibly with molecular oxygen. Like hemoglobin itself, the iron of the hemochrome upon oxygenation remains in the ferrous condition, although as a result of repeated cycling, it slowly oxidizes. The quantity of oxygen bound to the complexes approaches one mole per atom of iron, deviations probably being due to impurities. The work has been extended to a liquid solution of imidazole in pyridine. Under these conditions, both imidazole proto- and mesohemochromes indicate spectroscopically their combination with molecular oxygen.

Hemoglobin possesses the property of combining reversibly with molecular oxygen without oxidation of its iron from the ferrous to the ferric state. The complexity of the protein portion of this molecule has rendered studies on the relationship between structure and properties very difficult. However, simplified synthetic models can be used to throw light upon this relationship. Corwin and Erdman[2] observed that passivity with respect to oxidation by atmospheric oxygen can be secured by the exclusion of coördinating substances, such as water, which provide an electron transfer mechanism. Corwin and Reyes[1] found that crystalline di-imidazole protohemochrome combines reversibly with molecular oxygen in the absence of water, thus establishing the double linkage to imidazole as a sufficient condition for oxygenation in the crystalline state. This work was very laborious, however, and an improved method of preparation was indicated. The object of the present undertaking was to extend this work to other iron–porphyrin complexes and to liquid solutions.

Results.—From Table I it can be seen that *both* imidazole protohemochrome and imidazole mesohemochrome combined reversibly with molecular oxygen when subjected to oxygenation in the crystalline state. The amount of oxygen bound by these compounds approached one mole per mole of the heme. The purity of the compounds had a marked effect on their ability to combine reversibly with oxygen. Compounds 1, 2, 5 and 6 were less pure, containing excess imidazole as shown by carbon and hydrogen determinations. The maximum uptake by these less pure compounds did not exceed 0.88 mole per mole of heme. Those compounds which were of higher purity combined with a maximum of 0.96 mole of oxygen per mole of heme.

TABLE I

OXYGEN UPTAKE PER MOLE[a]

No.	Hemochrome	Cycle I		II		III	
		%	Mole	%	Mole	%	Mole
1	Meso[b]	3.45	0.85	3.28	0.81	2.15	0.53
2	Meso[b]	3.53	.87	3.16	.78	2.51	.62
3	Meso[c]	3.63	.89	3.44	.84	2.27	.55
4	Meso[c]	3.73	.92	3.08	.76	2.50	.64
5	Proto[b]	3.59	.88	1.83	.45	..	..
6	Proto[b]	3.02	.74	2.08	.51	0.73	0.18
7	Proto[c]	3.70	.91	3.18	.78	1.18	.29
8	Proto[c]	3.90	.96	3.38	.83	1.92	.47

[a] Based on one mole of O_2 per atom Fe. Calcd. for mesohemochrome, 4.06%; calcd. for protohemochrome, 4.08%. [b] Oxygenation, dry air, 8 hours, room temperature; desorption at 60–65°, 5 mm., 6 hours, under nitrogen. [c] Oxygenation, dry air, 8 hours, room temperature; desorption, 55–60°, 5 mm., 6 hours, under nitrogen.

It was also possible to follow the changes due to oxygenation spectroscopically. Table II summarizes these results.

(1) Porphyrin Studies. XV. Paper XIV, A. H. Corwin and Z. Reyes, THIS JOURNAL, **78**, 2437 (1956).

(2) A. H. Corwin and J. G. Erdman, *ibid.*, **68**, 2473 (1946).