radioactivity is much greater than the final activity of the benzyllithium. Indeed, for the same reason we may conclude that no appreciable amount of exchange occurs between 1,1,3-triphenylpropyllithium and benzyllithium; that is, essentially no exchange occurs after rearrangement. Also, no major amount of exchange takes place (necessarily reversible) between 2,2,3-triphenylpropyllithium and benzyllithium prior to rearrangement. We must therefore conclude that an irreversible exchange of benzyl groups in the reactants occurs during rearrangement itself. The radioactivity data are in good quantitative agreement with this conclusion and the additional conclusion that the molar activity of the 1,1,3-triphenylpropyllithium being formed at any specific time is identical with that of the benzyllithium in the solution at the same time (*i.e.*, a negligible isotope effect occurs). The molar activities of 2,2,4-triphenylbutanoic acid and phenylacetic acid calculated[9] on the basis of these conclusions and the initial quantities and activity of reactants are 1.20 and 0.335 μc./mmole, respectively. From these data it is obvious that the rearrangement of 2,2,3-triphenylpropyllithium is an *intermolecular* reaction. The elimination–readdition mechanism, involving elimination of benzyllithium followed by its readdition to 1,1-diphenylethene, fits all of the known data satisfactorily, provided that the benzyllithium which re-adds comes from the surrounding solution.

In a similar experiment phenyllithium (8.9 mmoles in 150 ml. of tetrahydrofuran), prepared from chlorobenzene[10] uniformly labeled with carbon-14, was added to 460 ml. of a tetrahydrofuran solution containing 15.0 mmoles of 2,2,2-triphenylethyllithium, all solutions[8] initially being at $-60 \pm 5°$. The resulting solution was allowed to warm with stirring to $5 \pm 5°$ and was maintained at this temperature for 4 hr. before carbonation. The results of this experiment are summarized in the following reaction sequence in which the activities of each compound assayed are given in units of microcuries per millimole.

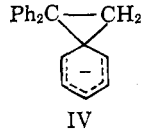
$$\underset{1.87}{C_6H_5Li} + (C_6H_5)_3CCH_2Li \longrightarrow C_6H_5Li + (C_6H_5)_2CLiCH_2C_6H_5$$

$$\downarrow CO_2$$

$$(C_6H_5)_2CCH_2C_6H_5 + C_6H_5CO_2H$$

$$\underset{<0.0009}{CO_2H} \qquad \underset{1.87}{}$$

The 2,2,3-triphenylpropanoic acid obtained was indistinguishable in radioactivity from background radioactivity and it is accordingly estimated that less than 0.05% (if any) of the activity of the starting phenyllithium could have become incorporated in the 2,2,3-triphenylpropanoic acid. On the basis of the present test, therefore, the migration of phenyl could be said to take place (as previously supposed[3-5]) by an *intramolecular* process, suitably by way of the transition state or reaction intermediate IV.[4] This is not a necessary,



IV

(9) These assumptions give rise to the expression $dx/dy = (A - x)/B$, where x and y are the number of microcuries and millimoles, respectively, of 1,1,3-triphenylpropyllithium formed at termination of reaction. A and B are the number of microcuries and millimoles, respectively, of benzyllithium at the start of reaction. Integration of this expression gives $y = 2.303B \cdot \log [A/(A - x)]$. Since rearrangement is complete under the conditions of the present experiment, y is taken to be equal to the number of millimoles of 2,2,3-triphenylpropyllithium initially present. The quantity of benzyllithium is assumed to remain constant throughout the reaction.

(10) H. Gilman and T. S. Soudy, *J. Org. Chem.*, **22**, 565 (1957).

explanation, however. An alternative mechanism is that while 2,2,2-triphenylethyllithium undergoes elimination of phenyl anion to give 1,1-diphenylethene, the phenyl anion and 1,1-diphenylethene exist in a solvent "cage" and recombine before radioactive phenyllithium can diffuse into the "cage." Future experiments are planned to try to distinguish between these and other mechanistic possibilities.

Acknowledgments.—Grateful acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this work. We also wish to thank Dr. C. F. Koelsch for encouraging us to undertake the present isotopic tracer experiments.

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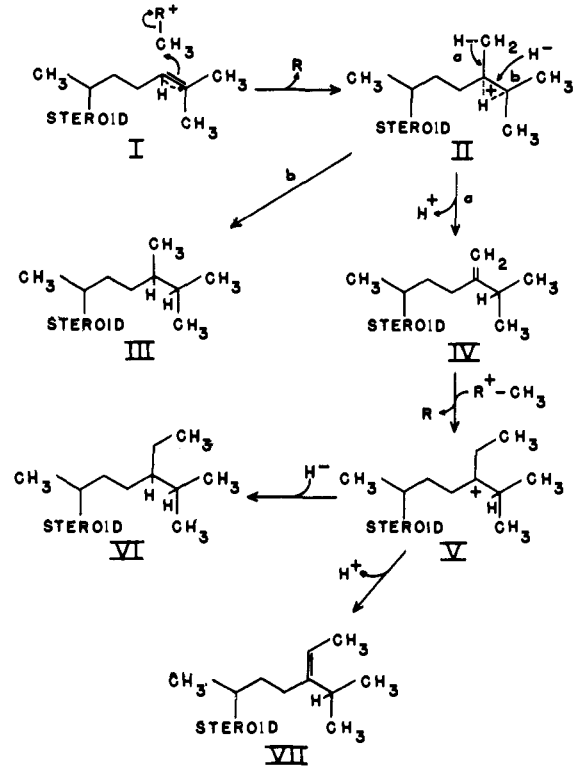
RECEIVED AUGUST 19, 1963

Evidence for the Origin of the Ethyl Group of β-Sitosterol[1]

Sir:

Mechanistic considerations which are implicit in the accompanying formulas led us to believe that the C_2 unit at position-24 of β-sitosterol (VI) might be derived from two C_1-units, and we should like to present experimental evidence in favor of this hypothesis.



Twenty seeds of *Pisum sativum* (the pea) were germinated for 4 days in water and then for 1 day in a solution containing 86 mg. of nonradioactive sodium mevalonate and tracer amounts of L-methionine labeled with C^{14} at the methyl group. The neutral fraction after saponification of an acetone extract was chromatographed on alumina together with pure β-amyrin and

(1) This investigation was supported in part by Training Grant No. CRTY-5001 of the National Institutes of Health.

recrystallized commercial β-sitosterol. When labeled methionine was used, radioactivity precisely cochromatographed with the sterol, and several recrystallizations failed to remove it. On the other hand, radioactivity only approximately cochromatographed with β-amyrin, and several recrystallizations almost completely removed labeled material. For example, 7.5×10^5 c.p.m. (0.032 mg.) of methionine led to 4550 c.p.m. which cocrystallized with β-sitosterol and only 55 c.p.m. which cocrystallized with β-amyrin. From another methionine incubation the β-amyrin band (Al_2O_3 chromatography, no carriers) was submitted to g.l.c. with continuous recording of radioactivity in the effluent (for conditions see next paragraph); two radioactive bands and one shoulder were observed, none of which coincided with the principal mass of the sample. The principal mass had the retention time of β-amyrin; the principal radioactivity had a retention time 132% as large as that of β-amyrin, and the secondary radioactive band had a retention time 110% as large as that of β-amyrin. By contrast, when 2-C^{14}-mevalonate was the radioactive precursor, similar g.l.c. analysis of the β-amyrin band (from Al_2O_3 chromatography) showed that only a single radioactive substance was present, and it had the same retention time as β-amyrin.

Since commercial β-sitosterol is contaminated with other steroids, *e.g.*, 5–31% of campesterol,[2] which are difficult to remove by recrystallization, we submitted the radioactive steroid (derived from methionine and separated from β-amyrin by chromatography on alumina) to g.l.c. The conditions used (at 230° using 0.92% nitrile silicone gum XE-60 on Anachrom AB, 90–100 mesh, treated with dimethyldichlorosilane) allowed the separation of sterols differing only by the presence of a C_1- or C_2-unit at C-24, *e.g.*, brassicasterol–stigmasterol and campesterol–sitosterol.[3] The 24-C_1 compounds moved 78% as fast as the 24-C_2 compounds. The effluent was passed through an ion chamber, and the radioactivity was continuously recorded. The principal radioactive band had the same retention time as β-sitosterol. An aliquot of the sample was also subjected to g.l.c. under the same conditions except that fractions were collected. The effluent equivalent to the β-sitosterol band was rechromatographed (g.l.c.) twice yielding a sample which showed only the presence of β-sitosterol on a fourth pass through g.l.c. This sample was cocrystallized with a sample of pure β-sitosterol.[4] Of 2840 c.p.m. collected which were cocrystallized with the carrier β-sitosterol until the specific activity was unchanged, 2430 c.p.m. were inseparable from the carrier.

Since we were unable to remove radioactivity from the β-sitosterol, it seems highly probable that the methyl C-atom of methionine was incorporated into this substance. It has been shown previously in this Laboratory that during the first 5 days radioactivity proceeding along the mevalonate pathway in germinating seeds of *Pisum sativum* is found predominantly in β-amyrin after the first day.[5-8] Since there was practically no β-amyrin produced from labeled methionine on the fourth to fifth day,[8] the radioactivity incorporated into the steroid cannot reasonably have arisen *via* the mevalonate route; consequently, carbon atoms numbered 1 through 27 inclusive should not be labeled.[9] This leaves only C-28 and C-29 as reasonable positions for the radioactivity in agreement with the mechanism shown in the formulas.

That a high degree of specificity exists in the transfer of a C_2-unit was shown by similar experiments with ethyl-labeled L-ethionine and with acetyl-labeled acetyl coenzyme-A. Both of these substrates proved to be very much less efficient precursors than L-methionine.

Recently Nicholas and Moriarty[10] reported that in *Salvia officinalis* β-sitosterol is produced with an increased percentage of label in the side chain (compared to the nucleus) when methionine (or formate) was the substrate than when either L-ethionine or mevalonate were substrates. Their results are in agreement with the sequence of events we propose in the accompanying formulas.

The formation of an ethyl group from two C_1-units by the route shown in the formulas is also supported by the presence of IV in nature (represented by 24-methylenecholesterol, sometimes called ostreasterol or chalinasterol)[11] and the presence of its analog (VII) with a 24-ethylidene group (represented by fucosterol)[11] which is reasonably derived from V by elimination of a proton. Furthermore, peas contain a minor component which is faster moving (g.l.c.) than is β-sitosterol, and its retention time (78% that of β-sitosterol) is compatible with a steroid III possessing a C_1-unit at C-24, *e.g.*, 22-dihydrobrassicasterol, if we assume the stereochemistry of reduction is the same as for the formation of β-sitosterol. When either methionine or mevalonate was the labeled substrate, fractions containing this faster moving component were radioactive both by continuous measurement of C^{14} in the g.l.c.-effluent and by fraction collecting followed by planchette counting.

The route proposed leading through a 24-methylene steroid (IV) is consistent with the observations of Fagerlund and Idler[12a] that in clams cholesterol can give rise to 24-methylenecholesterol, since they have shown[12b] that in the same organism cholesterol is dehydrogenated in the side chain with the introduction of a Δ^{22}- or a Δ^{25}-bond. Migration of the latter to the 24-position or direct introduction of a Δ^{24}-bond would give I which is also immediately available from the cyclization of squalene.[13] Our data, however, are equally compatible with the proposal advanced by Tschesche and Korte[14] that a $\Delta^{22,25}$-diene (rather than a Δ^{24}-monoene) is the intermediate which is alkylated.

While S-adenosyl methionine may be an intermediate in the alkylation as it appears to be in ergosterol

(2) M. J. Thompson, S. J. Louloudes, W. E. Robbins, J. A. Waters, J. A. Steele, and E. Mosettig, *Biochem. Biophys. Res. Commun.*, **9**, 113 (1962).

(3) We wish to thank Mr. J. M. Mercille for the preparation of brassicasterol from ergosterol by a modification of the route devised by D. H. R. Barton and C. H. Robinson, *J. Chem. Soc.*, 3045 (1954), and Mr. M. J. Thompson and Dr. A. Kuksis for the gift of authentic samples of campesterol.

(4) The β-sitosterol was prepared by the method of J. A. Steele and E. Mosettig, *J. Org. Chem.*, **28**, 571 (1963), from stigmasterol.

(5) D. J. Baisted, E. Capstack, and W. R. Nes, *Biochemistry*, **1**, 537 (1962).

(6) E. Capstack, D. J. Baisted, W. W. Newschwander, G. Blondin, N. L. Rosin, and W. R. Nes, *ibid.*, **1**, 1178 (1962).

(7) D. J. Baisted and W. R. Nes, *J. Biol. Chem.*, **238**, 1947 (1963).

(8) Unpublished observations by Dr. W. W. Newschwander in this Laboratory have shown specifically that on the 4th to 5th day of germination at 20° the β-amyrin to steroid ratio is greater than 1 for radioactivity incorporated from mevalonic acid. We have repeated this work under the precise conditions used for the methionine incubations, except that the amount of mevalonate was reduced; the fractions from the Al_2O_3-chromatogram were subjected to g.l.c. analysis, and, as expected, more radioactivity was present in β-amyrin than in β-sitosterol.

(9) This conclusion, of course, rests on the assumption that squalene is an intermediate to both β-amyrin and steroids.

(10) H. J. Nicholas and S. Moriarty, *Federation Proc.*, **22**, Part I, 529 (1963).

(11) For a discussion of the distribution of this sterol in nature and for a key to the literature, see W. Bergmann in M. Florkin and H. S. Mason, Ed., "Comparative Biochemistry," Vol. III, Academic Press, New York, N. Y., 1962, pp. 103–162.

(12) (a) U. H. M. Fagerlund and D. R. Idler, *Can. J. Biochem. Physiol.*, **39**, 1347 (1961); (b) *ibid.*, **39**, 505 (1961).

(13) R. B. Woodward and K. Bloch, *J. Am. Chem. Soc.*, **75**, 2023 (1953); T. T. Tchen and K. Bloch, *ibid.*, **77**, 6085 (1955).

(14) R. Tschesche and F. Korte, *Angew. Chem.*, **64**, 633 (1952); *ibid.*, **65**, 81 (1953); *ibid.*, **66**, 32 (1954); R. Tschesche, *Fortschr. Chem. Org. Naturstoffe*, **12**, 142 (1955).

synthesis,[15] this point remains to be defined for β-sitosterol.

(15) L. W. Parks, *J. Am. Chem. Soc.*, **80**, 2023 (1958).

(16) Postdoctoral trainee in the training program for Steroid Biochemistry.

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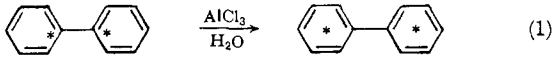
WILLIAM R. NES

RECEIVED MAY 16, 1963

The Rearrangement of Diphenyl[1]

Sir:

We wish to report unequivocal evidence for the water promoted, aluminum chloride induced intramolecular rearrangement of the benzene rings in diphenyl

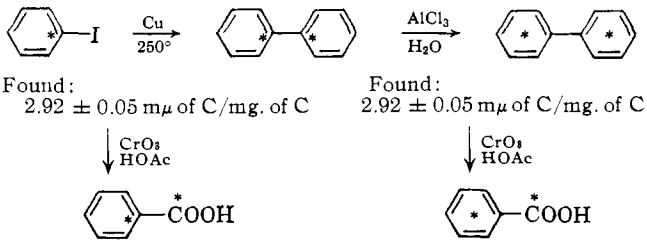


(1)

When diphenyl-1,1′-C^{14}, prepared in 80% yield *via* an Ullmann reaction[2] on iodobenzene-1-C_1^{14}, was heated to 100° for 30 min. with 10 mole % of aluminum chloride and 1 mole % of water, the radioactivity, originally localized at the two connecting carbons, had been randomly distributed. Recovered active diphenyl was also shown to be randomized when the reaction was carried out for 12 hr. in a refluxing benzene solution. The degradation method used is outlined in Scheme I.

SCHEME I

SYNTHESIS AND DEGRADATION OF ACTIVE DIPHENYL



Found:
2.92 ± 0.05 mμ of C/mg. of C

Found:
2.92 ± 0.05 mμ of C/mg. of C

Calcd.
for no rearrangement:
4.99 mμ of C/mg. of C

Found:
4.95 ± 0.06 mμ of C/mg. of C

Calcd.
for complete rearrangement:
2.92 mμ of C/mg. of C

Found:
2.92 ± 0.05 mμ of C/mg. of C

The view that the reaction is intramolecular[3] is supported by the following facts: (1) The inactive benzene used in the solvent experiments was devoid of activity at the end of a run within the precision of our assay methods.[4] (2) A rearrangement carried out with inactive diphenyl in benzene-1-C_1^{14} yielded diphenyl having an activity indicating less than 0.001% intermolecularity.

The isomerizations in benzene were carried out by refluxing 0.5 g. of diphenyl-1,1′-C^{14} in 10 ml. of benzene containing 50–100 mg. of aluminum chloride and 5–10 mg. of water for various periods of time. The diphenyl, isolated after quenching the reaction mixture and evaporating the benzene, was oxidized[5] by stirring for 5 hr. at 40–50° with 3.0 g. of chromium trioxide in 35 ml. of glacial acetic acid, furnishing benzoic acid in 35–47% yields.

As a secondary check on the exchange, radioactive benzene alone was treated with the aluminum chloride–water catalyst. Carrier diphenyl was added after the reaction had been quenched. The isolated and purified diphenyl had an activity indicating a benzene to diphenyl conversion of less than 0.01%.[6]

Our experimental data do not allow us to distinguish between a hydrogen abstraction[7] mechanism and a proton addition[8] mechanism to develop the positive charge on the *ortho* carbon. The latter pathway followed by a 1,2-shift and proton loss seems to us the simplest explanation, although other mechanisms cannot be excluded at this stage. If a proton addition mechanism is operative during this facile rearrangement of diphenyl in the presence of a water promoted Lewis acid, some doubt is thrown on the stability of polyaryls[9] under conditions of electrophilic substitution reactions. Furthermore it seems to us that the significance of isomer distribution in diphenyl[10] would require some re-examination.

(1) Research performed under the auspices of the U. S. Atomic Energy Commission.

(2) The benzoic acid obtained by oxidation of the unrearranged diphenyl-1,1-C^{14} showed no loss in specific activity. This indicated *inter alia* that the Ullmann reaction proceeds without rearrangement. The iodobenzene-1-C_1^{14} was prepared in 76% yield from aniline-1-C_1^{14} (*cf.* H. J. Lucas and E. R. Kennedy, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 351) supplied by Nuclear Research Chemicals, Inc., Orlando, Florida.

(3) G. A. Olah and M. W. Meyer, *J. Org. Chem.*, **28**, 1912 (1963).

(4) D. R. Christman, M. E. Day, P. R. Hansell, and R. C. Anderson, *Anal. Chem.*, **27**, 1935 (1955). The authors are indebted to Dr. D. R. Christman and Mrs. C. T. Paul for the activity assays.

(5) J. C. Colbert and C. L. Hensley, *J. Am. Chem. Soc.*, **62**, 3257 (1940).

(6) The yield of diphenyl from benzene in the absence of diphenyl may well be greater than in the presence of diphenyl, the latter presumably complexing better with the catalyst than benzene.

(7) A. Streitwieser, Jr., and L. Reif, *J. Am. Chem. Soc.*, **82**, 5003 (1960).

(8) R. H. Allen, *ibid.*, **82**, 4856 (1960); *cf.* H. C. Brown and C. R. Smoot, *ibid.*, **78**, 2176 (1956); H. Steinberg and F. L. J. Sixma, *Rec. trav. chim.*, **81**, 185 (1962); E. Ünseren and A. P. Wolf, *J. Org. Chem.*, **27**, 1509 (1962).

(9) P. Kovacic and F. W. Koch, *ibid.*, **28**, 1864 (1963).

(10) M. J. S. Dewar, T. Mole, D. S. Urch, and E. W. T. Warford, *J. Chem. Soc.*, 3572 (1956); L. M. Stock and H. C. Brown, *J. Am. Chem. Soc.*, **84**, 1242 (1962); R. Baker, R. W. Bott, and C. Eaborn, *J. Chem. Soc.*, 2136 (1963).

(11) Visiting professor at Brookhaven National Laboratory, June 14, 1963–September 4, 1963, from University of Groningen, Holland.

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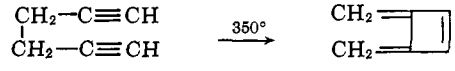
RECEIVED AUGUST 28, 1963

3,4-Dimethylenecyclobutene by Thermal Rearrangement of 1,5-Hexadiyne

Sir:

We have found that 1,5-hexadiyne (I) rearranges at 350° giving 3,4-dimethylenecyclobutene (II) in 85% yield. The reaction is conducted in a flow system, using



I II

a Pyrex tube (12 mm. × 65 cm.) packed with glass helices as the reaction vessel. The diyne is vaporized and swept through the reaction tube in a stream of nitrogen. It is important that the hydrocarbon be vaporized before it reaches the reaction zone. If the liquid is allowed to drop directly onto the helices, it ignites each time a drop hits, and after a short time a vigorous reaction occurs filling the apparatus with soot. This trouble is not experienced when the hydrocarbon is vaporized first. Complete conversion of I occurs at 350° using a hydrocarbon feed rate of 6 ml./hr. and a nitrogen flow of 2400 ml./hr. The v.p.c. tracing of the product shows a single peak.

The infrared and ultraviolet spectra are in agreement with those reported for II by Blomquist and Maitlis.[1] The boiling point which we observe (72°) is substan-

(1) A. T. Blomquist and P. M. Maitlis, *Proc. Chem. Soc.*, 332 (1961).