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Unsaturated Fatty Acids. IV. Preparation of Oleic-1-C^{14} Acid[1]

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By a series of reactions analogous to that employed earlier[2] in the preparation of linoleic-1-C^{14} acid, oleic-1-C^{14} acid has now been synthesized by radiocarbonation of the Grignard reagent of *cis*-1-bromo-8-heptadecene, which was obtained *via threo*-9,10-dibromooctadecanoic acid, its silver salt, and *threo*-1,8,9-tribromoheptadecane.

The acid was treated with diazomethane to give methyl oleate-1-C^{14}, which has been used in metabolism studies reported elsewhere.[3] This synthesis of oleic-1-C^{14} acid is to be compared with that reported recently by Bergström and coworkers,[4] which differs from that presently employed only in protection of the *cis*-double bond by hydroxylation (instead of bromination) and in introduction of the labeled carboxy carbon by a nitrile synthesis instead of by a Grignard carbonation. Although the Bergström synthesis involves four labeled-reactant steps, while isotope is introduced in the final step of the present method, the isotope yields realized are comparable—48[4] and 59%.

EXPERIMENTAL

Unless otherwise indicated, reactions were conducted as described in detail previously.[2] Ultimate analyses were performed by Dr. A. Elek (Elek Micro Analytical Laboratories, Los Angeles), infrared absorption analyses by Mr. Paul Kratz, and some of the radioactivity determinations by Mr. W. H. Slaton, Jr. All melting points are corrected. Reported *trans*-contents of olefinic substances, based on infrared absorption at 10.3μ, are considered to be within 5% of actual values.

Purified oleic acid was obtained[5] from commercial material (Merck U.S.P.) by fractional distillation, low-temperature crystallization (from 10% solution in acetone at −20 to −50°), and redistillation, b.p. 168–173° at about 200μ, water-white, m.p. 10°, n_D^{25} 1.4580; this material is expected to contain about 3% each of linoleic[6] and saturated acids,[5] all or part of which may persist as contaminants in the labeled oleic acid (see below).

threo-9,10-Dibromooctadecanoic acid (I). Although this substance has been employed in a great number of published investigations, in only one instance[7] has it been reported as a crystalline compound, m.p. 28.5–29°. Inasmuch as oleic acid is difficult to obtain in a high state of purity and since, moreover, addition of bromine to the acid is accompanied by some 8–10% of anomalous substitution reactions (giving rise to hydrogen bromide and bromine substituents in positions other than 9 and 10 of the stearic acid skeleton[8]), the reported crystallizability of I was of interest from the standpoint of suggesting an additional means of purification. By mixing equivalent amounts of oleic acid and bromine in carbon tetrachloride at 0°, a sample of I was obtained which crystallized spontaneously on standing neat for a short time at −10°. This material had a crude melting point of 20–24° and a refractive index (n_D^{42}) of 1.4878 (lit.[7] n_D^{42} 1.4878), and was shown by chromatography on alumina[9] of the methyl ester prepared from it with diazomethane to be essentially free of unbrominated and tetrabromide contaminants and by infrared absorption studies on the zinc-debrominated ester to be free of the *erythro*-9,10-dibromo-diastereoisomer.[10] But despite the apparent purity of the preparation, low-temperature recrystallization failed to result in good recovery of material of improved purity (as judged by melting point; repeated crystallization starting with 8.4 g. of crude I gave 0.32 g. of solid, m.p. 27–28°)[13]; consequently the crude product obtained under conditions designed to minimize anomalous substitution was employed.

In a nitrogen atmosphere, a solution of 28.2 g. (0.1 mole) of purified oleic acid and 0.28 g. of di-*t*-butyl-*p*-cresol (Koppers Co.) in 200 ml. of carbon tetrachloride was heated to boiling (to drive traces of moisture from the reactants and apparatus), cooled to 0°, and treated dropwise with dry bromine (16 g., 0.1 mole) over a period of about 30 min.;

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[1] This paper is based on work performed under Contract AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

[2] D. R. Howton, R. H. Davis, and J. C. Nevenzel, *J. Am. Chem. Soc.*, **76**, 4970 (1954).

[3] J. F. Mead, W. H. Slaton, Jr., and A. B. Decker, *J. Biol. Chem.*, **218**, 401 (1956).

[4] S. Bergström, K. Pääbo, and M. Rottenberg, *Acta Chem. Scand.*, **6**, 1127 (1952); the authors are indebted to Prof. Bergström for making available a manuscript of this work prior to its publication.

[5] D. Swern, H. B. Knight, and T. W. Findley, *Oil & Soap*, **21**, 133 (1944).

[6] Oleic acid having a lower linoleic acid content may be obtained from olive oil fatty acids by a similar procedure; cf. H. B. Knight, E. F. Jordan, Jr., E. T. Roe, and D. Swern, *Biochemical Preparations*, **2**, 100 (1952).

[7] D. Holde and A. Gorgas, *Z. angew. Chem.*, **39**, 1443 (1926).

[8] Unpublished observations.

[9] D. R. Howton, *Science*, **121**, 704 (1955).

[10] The apparently good stereospecificity of the bromination-zinc-debromination cycle as applied to oleic acid is in agreement with findings (based on less sensitive methods) of Nicolet[11] and of Holde and Gorgas,[7] although at odds with results obtained on simpler olefins[12]; and is of interest in connection with the rather appreciable *trans*-content of the reconstituted oleic acid obtained in the present study (see below).

[11] B. H. Nicolet, *J. Am. Chem. Soc.*, **43**, 2122 (1921).

[12] W. G. Young, S. J. Cristol, and T. Skei, *J. Am. Chem. Soc.*, **65**, 2099 (1943).

[13] The low crystallizability of I compared with that of the structurally and physically closely similar *erythro*-isomer (m.p. 29.5–30.0°, prepared in the same way from elaidic acid) is strikingly illustrated by cooling 10% solutions of the two substances in n-pentane in 5°-increments; the *erythro*-acid crystallizes out in good yield at −15°, while the *threo*-acid does not emerge in appreciable amount until the solution is cooled to −50°.

decolorization was rapid until about 90% of the bromine had been added. After stirring an additional 30 min. at 0°, the still-colored mixture was shaken with aqueous sodium bisulfite (discharging the color), then with water until free of mineral acid, and dried over magnesium sulfate. Solvent was stripped from the crude product (44.5 g.), which was dissolved in 450 ml. of acetone and stirred at −20° for 75 min., precipitating a small amount of solid (presumably bromine-free and tetrabromo contaminants), which was removed by filtration. Removal of solvent from the filtrate left 43.3 g. (98%) of crude I, viscous brown oil, which was employed directly in preparation of the silver salt (II).

Methyl threo-9,10-dibromooctadecanoate was prepared from the recrystallized sample of I (m.p. 27–28°) by treatment with excess ethereal diazomethane. The ester gave a copious precipitate on warming with ethanolic silver nitrate, and a perceptible cloudiness after only 15 min. at room temperature. The reactivity of the bromine substituents in this material, reflected in the instability of the silver salt of I (see below), is to be compared with that of those in methyl *threo,threo*-9,10,12,13-tetrabromooctadecanoate, which does not react detectably with silver nitrate in 15 hr. at room temperature.[2]

Silver threo-9,10-dibromooctadecanoate (II) was prepared from 0.1 mole of crude I as described earlier[2] with reference to the tetrabromo analog. The product, obtained in 93% crude yield, was a tan, slightly sticky powder; as indicated by increased stickiness and formation of silver bromide, the salt deteriorates on standing and hence should be used as soon after preparation as possible.

threo-1,8,9-Tribromoheptadecane (III) was prepared under conditions found[14] to give optimum yields of 1-bromoheptadecane from silver stearate. A slurry of 50.0 g. (0.091 mole) of II in dry carbon tetrachloride was cooled to 0° and treated while stirring with 96 g. of a 15.2-weight % solution of bromine in the same solvent (total bromine added: 14.6 g., 0.091 mole). About 15–20% of the total expected carbon dioxide was collected during this stage. The cooling bath was then replaced by a steam bath; gas evolution was complete by the time the solution reached the boiling point, with 75–85% of the theoretical quantity of carbon dioxide being evolved (including that collected at 0°).

The reaction products were separated by chromatography on a 7.4 (diam.) × 20.5 cm. column of silicic acid (J. T. Baker reagent powder) prewashed as described earlier[2] and developed with 1.75 column-volumes each of 60–70° petroleum ether, 30% (by volume) benzene in petroleum ether, and absolute ethyl ether; yields: 52–53% III, 6–7% ester, and 26–36% recovered acidic material. A rechromatographed sample of III (eluted with *n*-pentane) was a pale yellow oil, $n_D^{25.0}$ 1.5065, $d^{21.2}$ 1.384.

Anal. Calcd. for $C_{17}H_{33}Br_3$: C, 42.78; H, 6.97; Br, 50.24. Found: C, 42.89; H, 6.75; Br, 50.22.

cis-1-Bromo-8-heptadecene (IV). The debromination of 10.4 g. (0.021 mole) of III in 20 ml. of benzene with 8.0 g. (0.12 mole) of activated 20-mesh granulated zinc in 25 ml. of ethanol proceeded readily, as judged by spontaneous reflux during the addition of the tribromide, and was completed by heating for an additional 15 min. Distillation of the product gave 5.65 g. (85%) of faintly yellow oil, b.p. 136–146° at 0.2 mm., the latter 4.55 g. of which (b.p. 140–146°) was reserved for subsequent steps and characterization; n_D^{25} 1.4704, d^{24} 1.018, infrared absorption at 10.3μ indicative of the presence of about 20% of the *trans*- isomer of IV.[15]

Anal. Calcd. for $C_{17}H_{33}Br$: C, 64.34; H, 10.48; Br, 25.18. Found: C, 64.20; H, 10.59; Br, 25.07.

Some improvement in the geometric homogeneity of IV was realized by low temperature crystallization from acetone (20% solutions), the higher melting, less soluble *trans*-material being concentrated in the precipitates, which were discarded. Thus 8.3 g. of crude IV (25% *trans*) after 5 hr. at −40° gave 7.2 g. 20% *trans;* a second application of the procedure (1 hr. at −60°) gave 4.74 g. 10% *trans*, b.p. 134.0–136.5° at 180 μ, n_D^{25} 1.4708, which was employed in preparation of the Grignard reagent (see below).

trans-1-Bromo-8-heptadecene was obtained by similar low temperature crystallization of a sample of crude IV (40% *trans*) prepared from a commercial sample of oleic acid later shown to contain rather large amounts of elaidic acid. The crystalline bromide (m.p. −9°, b.p. 115–125° at 100–125μ, n_D^{25} 1.4691, d^{27} 1.001) gave an infrared spectrum indicating 95% *trans*- component.

Oleic-1-C^{14} acid. Magnesium turnings (97.1 mg., 4.00 mmoles) were dried in the reaction vessel[2] by pumping down to 0.03μ, and the system was then filled with dry, oxygen-free nitrogen (tank gas passed through Fieser's solution,[17] Drierite, and a liquid nitrogen trap). The stirrer was started and a few milliliters of a solution of 1.1662 g. (3.67 mmoles) of IV (10% *trans*) and 73.1 mg. (0.51 mmoles) of methyl iodide in 27 ml. of freshly distilled, dry ethyl ether was added. With the vessel at room temperature the solution became hazy in 5–6 min., indicating that reaction had started; the remaining halide solution was then added dropwise over a 90-min. period. Stirring was continued for an additional 30 min. After the mixture had been let stand overnight, titration of a 1-ml. aliquot indicated formation of 3.47 mmoles of Grignard (83% on the basis of total halide employed). Essentially as described in detail earlier,[2] the remaining Grignard reagent (3.31 mmoles) was carbonated with the carbon dioxide from 746.1 mg. (3.782 mmoles) of barium carbonate containing 2.06 mc. C^{14}.

The reaction mixture was acidified and the products were taken up in *n*-pentane, washed with water, and freed of solvent, leaving a viscous yellow oil (0.98 g.) which was chromatographed on silicic acid to yield (in order of elution) 0.185 g. of hydrocarbon (presumably 9,25-tetratriacontadiene, formed from IV by a Wurtz-type reaction accompanying Grignard formation[2]), 0.025 g. of material of intermediate polarity, 0.65 g. of oleic-1-C^{14} acid (neut. equiv. 280; theory 282), and 0.06 g. of more strongly adsorbed material. The yield of oleic-1-C^{14} acid was 63.2% from IV (corrected for the aliquot of Grignard solution titrated) or 58.6% from BaC^{14}O$_3$.

Methyl oleate-1-C^{14} was prepared by treating the acid in ether with diazomethane; the crude product was purified by chromatography on a silicic acid column, yield 75%. The ester (counted as an "infinitely thick" sample after dilution with "cold" methyl oleate) had an activity of 1.71 mc./g. (calculated from the activity of BaC^{14}O$_3$ used as starting material, 1.84 mc./g.).

Because of the nature of the metabolism studies in which this ester was to be used, it was important to determine its content of methyl linoleate-1-C^{14} with some precision. A small sample of the ester which had been diluted about 3:1 with corn oil (for feeding experiments) was diluted further with about 10 parts of "cold" methyl linoleate,[18] and saponified to

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(14) J. C. Nevenzel and D. R. Howton, unpublished.

(15) In view of the demonstrated good stereospecificity of the bromination-zinc-debromination of oleic acid[10] and the fact that elaidinization of linoleic acid (or of linolenic acid[14]) during an analogous decarboxylation-reconstitution[2] was not significantly greater than that routinely observed in its bromination-zinc-debromination, the rather extensive elaidinization of the *cis*-double bond in the present instance is noteworthy and probably attributable to the greater reactivity of the bromine substituents of its precursors. Racemization of the *threo*-dibromo grouping to *erythro* during treatment of the silver salt (II) with bromine is suspect; racemization of a reactive alkyl bromide under such circumstances has been reported.[16]

(16) C. L. Arcus, A. Campbell, and J. Kenyon, *J. Chem. Soc.*, 1510 (1949).

(17) L. F. Fieser, *Experiments in Organic Chemistry*, Part II, 2nd Ed., D. C. Heath and Co., New York, 1941, pp. 395–396.

(18) *Cf.* J. W. McCutcheon, *Org. Syntheses*, 22, 75 (1942).

yield a mixture of fatty acids having a specific activity of 1695 d./sec./mg. Bromination of a 98.7-mg. sample of the fatty acid mixture in 3.5 ml. of *n*-pentane according to White and Brown[19] gave 83.85 mg. of crude *threo,threo*-9,10,12,13-tetrabromooctadecanoic acid (TBS) and showed the mixture to contain 89% linoleic acid. (Preliminary experiments with corn oil fatty acids and with linoleic acid[18] gave 65 and 95% linoleic acid, respectively, in good agreement with the expected values.) The crude TBS and successive recrystallized (from acetone) samples had specific activities of 92.1, 23.0, 18.3, and 17.0 d./sec./mg., the last corresponding to a linoleic acid content in the oleic-1-C^{14} acid of 1.90%.

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[19] M. F. White and J. B. Brown, *J. Am. Oil Chem. Soc.*, **26**, 385 (1949).

Diels-Alder Reactions of *o*-, *m*-, and *p*-Nitrostyrene[1]

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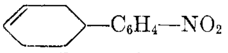
The electrophilic properties of 2- and 4-vinylpyridine and of the electronically similar *o*- and *p*-nitrostyrene have been amply verified in recent years. Thus, it has been shown that these compounds readily undergo Michael-type addition at the double bond with a wide variety of nucleophiles.[2] That this behavior may be traced directly to the conjugation of the ethylenic linkage with the electronegative imino or nitro group is indicated by the lack of reactivity of 3-vinylpyridine and of *m*-nitrostyrene under the same conditions.[2a-c]

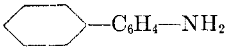
Although a detailed description of the mechanism of the Diels-Alder reaction is still lacking, it is generally assumed that the activity of the dienophile may be correlated with the degree of activation of the olefinic bond by electronegative groups acting through conjugation.[3] In view of these facts, it seemed reasonable to expect enhanced dienophilic properties in those vinyl aromatics for which electrophilic properties had been demonstrated.[2] Doering and Rhoads explored this possibility with 2- and 3-vinylpyridine[4]; their results, while confirming the activity of 2-vinylpyridine, also revealed 3-vinylpyridine as an equally effective dienophile. In order to test the generality of this lack of differentiation among position isomers, the study has now been extended to the nitrostyrene series.[5]

So that a direct comparison might be made in this series, a standard set of reaction conditions was used. Butadiene served as the reference diene in all cases. Preliminary runs indicated that a reaction temperature of 125° and a mole ratio of diene to dienophile of *ca.* 2.5 to 1 brought about appreciable reaction over a period of 20 to 40 hr. while minimizing the concurrent polymerization. Even so, the latter was appreciable and necessitated the development of isolation procedures which would assure the best yields of adducts. The thermal stability of the adducts under the reaction conditions was examined by heating them alone at 125° or higher for 40 hr.

The reaction of *p*-nitrostyrene and butadiene proceeded readily under the reaction conditions to yield the adduct, 4-(*p*-nitrophenyl)cyclohexene (Ia). The structural assignment is based on analysis and on reduction of the adduct to the known *p*-aminophenylcyclohexane (IIa). In Ia, as in the other adducts, the position of the double bond is assigned by analogy to the usual Diels-Alder result.



I a: *p*-nitro
b: *o*-nitro
c: *m*-nitro



II a: *p*-amino
b: *o*-amino
c: *m*-amino

Under the same conditions, *o*-nitrostyrene added butadiene to form 4-(*o*-nitrophenyl)cyclohexene (Ib). The latter, a low melting solid, was characterized as its dibromide, III. Catalytic reduction of Ib gave the known *o*-aminophenylcyclohexane (IIb).

m-Nitrostyrene has been prepared from the corresponding nitrocinnamic acid by a modification of the method of Wiley and Smith.[6a] By using a flash distillation technique[6b] to remove the styrene from the reaction flask, yields of 88% have been realized. The reaction of *m*-nitrostyrene and butadiene yielded the expected addition product, 4-(*m*-nitrophenyl)cyclohexene (Ic). On reduction, Ic absorbed four mole equivalents of hydrogen producing *m*-aminophenylcyclohexane (IIc), characterized as the benzenesulfonamide.

Under the standard reaction conditions of 40 hr. at 125°, the yields of purified, stable adducts, Ia, Ib, and Ic, were 60, 48, and 44%, respectively. Although such data are admittedly crude, it seems

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[1] Abstracted from the M. S. theses of C. B. H. and V. M. H.

[2] (a) W. von E. Doering and R. A. N. Weil, *J. Am. Chem. Soc.*, **69**, 2461 (1947); (b) H. B. Hass and M. L. Bender, *J. Am. Chem. Soc.*, **71**, 3482 (1949); (c) W. J. Dale and C. W. Strobel, *J. Am. Chem. Soc.*, **76**, 6172 (1954); (d) W. J. Dale and G. Buell, *J. Org. Chem.*, **21**, 45 (1956).

[3] K. Alder, C. V. Wilson, and J. A. VanAllan in *Newer Methods of Preparative Organic Chemistry*, Interscience Publishers, Inc., New York, N. Y., 1948, p. 399.

[4] W. von E. Doering and S. J. Rhoads, *J. Am. Chem. Soc.*, **75**, 4738 (1953).

[5] N. C. Deno and J. D. Johnston [*J. Am. Chem. Soc.*, **74**, 3233 (1952)] have reported the reactions of *o*-, *m*-, and *p*-nitrostyrene with sorbic acid, but under reaction conditions which make assessment of relative reactivity difficult.

[6] (a) R. H. Wiley and N. R. Smith, *J. Am. Chem. Soc.*, **70**, 2295 (1948); (b) *cf.* R. H. Wiley and M. H. Hobson, *ibid.*, **71**, 2429 (1949) who developed this technique for the preparation of the thermally sensitive *p*-formylstyrene.