Synthesis of Methyl 5,8,11,14-Eicosatetraenoate (Methyl Arachidonate)[1,2]

By Seyhan N. Ege, Reuven Wolovsky[3] and Walter J. Gensler

Received February 10, 1961

Arachidonic acid as its methyl ester has been synthesized via acetylenic intermediates. The Grignard derivative of 1-heptyne was coupled with 4-chloro-2-butyn-1-ol to give 2,5-undecadiyn-1-ol, which with phosphorus tribromide gave 1-bromo-2,5-undecadiyne. 8-Chloro-1,4-octadiyne was obtained from the 5-chloro-1-pentynyl Grignard reagent and propargyl bromide. Coupling 1-bromo-2,5-undecadiyne with 8-chloro-1 4-octadiyne yielded the tetraacetylene 1-chloro-4,7,10,13-nonadecatetrayne. Semi-hydrogenation followed by carbonation of the Grignard derivative offered arachidonic acid which upon esterification with diazomethane gave methyl arachidonate.

The group of essential fatty acids includes linoleic acid (all cis-9,12-octadecadienoic acid), γ-linolenic acid (all cis-6,9,12-octadecatrienoic acid) and arachidonic acid (all cis-5,8,11,14-eicosatetraenoic acid). These acids have long been recognized[4] as dietary factors in the normal growth of animals. It has generally been accepted[4-6] that the all cis configuration and the skipped[7] arrangement of double bonds are essential to the biological activity of these acids. More recently, through their action in depressing blood cholesterol levels, they have attracted attention as potential agents for the control of atherosclerosis.[4] Arachidonic acid, the most potent of the group,[8] seems to be, therefore, of great importance as a nutritional factor.

Since arachidonic acid is not easily available in quantity from natural sources, it seemed desirable to develop a practical route for its synthesis. When we started our work, several syntheses of linoleic acid had been described[9] but none of arachidonic acid.[10] We are now reporting a practical synthesis of arachidonic acid.

Combination of two diunsaturated fragments to form a tetraunsaturated skipped system such as in arachidonic acid offers some advantage over combination of a triunsaturated with a monounsaturated fragment. This is a consequence of the

sharply increasing difficulty in handling sensitive skipped unsaturated compounds as the number of unsaturations goes up. With this in mind, the scheme of synthesis outlined in Fig. 1 was developed. The two fragments, 1-bromo-2,5-undecadiyne (IV) and 8-chloro-1,4-octadiyne (VI), were synthesized and coupled to give 1-chloro-4,7,10,13-nonadecatetrayne (VII)[11] which is a key intermediate to arachidonic acid.

In carrying out the coupling reactions of acetylenic Grignard reagents with propargylic halides, the use of tetrahydrofuran as a solvent was found to be of great advantage. Preliminary experiments on the cuprous chloride-catalyzed coupling of 1-heptyne with propargyl bromide, as well as of 5-chloro-1-pentyne with propargyl bromide in ether, in tetrahydrofuran at 37°, and in tetrahydrofuran at 65°, indicated that the best results were obtained when tetrahydrofuran was used as solvent around room temperature. From these experiments two effects were apparent. First, reaction times were cut from periods of several days to about one to two hours when tetrahydrofuran was used instead of ether at 37°. Second, higher temperatures in tetrahydrofuran were not of further advantage since lower yields resulted as a consequence of decomposition or side reactions.[12]

2,5-Undecadiyn-1-ol (III), obtained in 87% yield from the reaction of 4-chloro-2-butyn-1-ol (II)[13] with the Grignard derivative of 1-heptyne, was converted in 70% yield to the corresponding bromide IV by treatment with phosphorus tribromide. 8-Chloro-1,4-octadiyne (VI) was obtained in 53% yield by coupling the Grignard reagent from 5-chloro-1-pentyne (V) with propargyl bromide. The cuprous chloride-catalyzed coupling of the Grignard derivative of the chlorodiyne VI with 1-bromo-2,5-undecadiyne (IV) in cold tetrahydrofuran yielded 1-chloro-4,7,10,13-nonadecatetrayne (VII) in 65% yield. The tetrayne VII, upon careful semi-hydrogenation over Lindlar catalyst[14] in the presence of quinoline, followed

(1) For the previous paper in the series on the synthesis of unsaturated fatty acids, see W. J. Gensler and C. B. Abrahams, J. Am. Chem. Soc., **80**, 4593 (1958).

(2) This investigation was supported by a research grant (No. H3773) from the National Heart Institute, U. S. Public Health Service.

(3) Visiting Scientist from the Weizmann Institute of Science, Rehovoth, Israel, 1959-1960.

(4) For a comprehensive review and leading references, see H. J. Deuel, Jr., "The Lipids," Vol. III, Interscience Publishers, Inc., New York, N. Y., 1957, p. 783.

(5) T. P. Hilditch, "The Chemical Constitution of Natural Fats," Third Edition, John Wiley and Sons, Inc., New York, N. Y., 1956, Chapter IX, pp. 483-550.

(6) R. T. Holman, Proc. Soc. Exptl. Biol. Med., **76**, 100 (1951).

(7) The term "skipped" designates unsaturations separated by a single methylene group in the same way as the descriptive meaning of "conjugated" serves for unsaturations separated by a single bond. See W. J. Gensler and A. P. Mahadevan, J. Am. Chem. Soc., **77**, 3076 (1955).

(8) S. M. Greenberg, C. E. Calbert, H. J. Deuel, Jr., and J. B. Brown, J. Nutrition, **45**, 521 (1951).

(9) C. R. Noller and M. C. Girvin, J. Am. Chem. Soc., **59**, 606 (1937); P. Baudart, Bull. soc. chim., [5] **11**, 336 (1944); R. A. Raphael and F. Sondheimer, J. Chem. Soc., 2100 (1950); H. M. Walborsky, R. H. Davis and D. R. Howton, J. Am. Chem. Soc., **73**, 2590 (1951); W. J. Gensler and G. R. Thomas, ibid., **73**, 4601 (1951); I. K. Sarycheva, G. I. Myagkova and N. A. Preobrazhenskii, Zhur. Obshchei Khim., **29**, 2318 (1959) [C. A., **54**, 9744 (1960)].

(10) In the meantime two communications on the synthesis of arachidonic acid have appeared: (a) J. M. Osbond and J. C. Wickens, Chemistry & Industry, 1288 (1959); (b) M. W. Goldberg and A. I. Rachlin, U. S. Patent 2,934,570 (Apr. 26, 1960) [C. A., **54**, 18358 (1960)].

(11) Chlorotetrayne VII has also been prepared by the coupling of 1,4,7-tridecatriyne with 1-bromo-6-chloro-2-hexyne by V. S. Gandhi (unpublished work) in our laboratories as well as by Goldberg and Rachlin.[10b]

(12) For instance, coupling of 1-heptyne with propargyl bromide in tetrahydrfouran yielded, besides the normal coupling product, 1,4-decadiyne, CH₃(CH₂)₄C≡CCH₂C≡CH, ca. 23% of a higher boiling compound (probably 1,4,7-tridecatriyne, CH₃(CH₂)₄C≡CCH₂C≡CCH₂C≡CH) at reflux temperature and only ca. 3% at 37°. In addition, the product formed at reflux temperature contained considerably larger amounts of allenic impurities than the one obtained at 37° as evidence by the bands at 1940 cm.⁻¹ in their infrared spectra.

(13) W. J. Bailey and E. Fujiwara, J. Am. Chem. Soc., **77**, 165 (1955).

$CH_3(CH_2)_4C{\equiv}CH$
I

$HC{\equiv}C(CH_2)_3Cl$
V

$\downarrow ClCH_2C{\equiv}CCH_2OH$
II

$\downarrow HC{\equiv}CCH_2Br$

$CH_3(CH_2)_4C{\equiv}CCH_2C{\equiv}CCH_2OH$
III

$HC{\equiv}CCH_2C{\equiv}C(CH_2)_3Cl$
VI

$\downarrow PBr_3$

$CH_3(CH_2)_4C{\equiv}CCH_2C{\equiv}C{\cdot}CH_2Br$ ————————————————
IV

$CH_3(CH_2)_4C{\equiv}CCH_2C{\equiv}CCH_2C{\equiv}CCH_2C{\equiv}C(CH_2)_3Cl$
VII

$CH_3(CH_2)_{18}Cl$ $H_2(Pt)$
IX

$\downarrow H_2$, Lindlar catalyst

$H_2(Pt)$

$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3Cl$
VIII (all *cis*)

1, Mg $\downarrow$ 2, CO_2

$CH_3(CH_2)_{18}COOH$ $\xleftarrow{H_2}{Pt}$ $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$
XI   X (arachidonic acid) (all *cis*)

$\downarrow CH_2N_2$

$CH_3(CH_2)_{18}COOCH_3$ $\xleftarrow{H_2}{Pt}$ $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOCH_3$
XIII   XII (methyl arachidonate) (all *cis*)

$\downarrow Br_2$

$CH_3(CH_2)_4CH{-}CHCH_2CH{-}CHCH_2CH{-}CHCH_2CH{-}CH{-}(CH_2)_3COOCH_3$
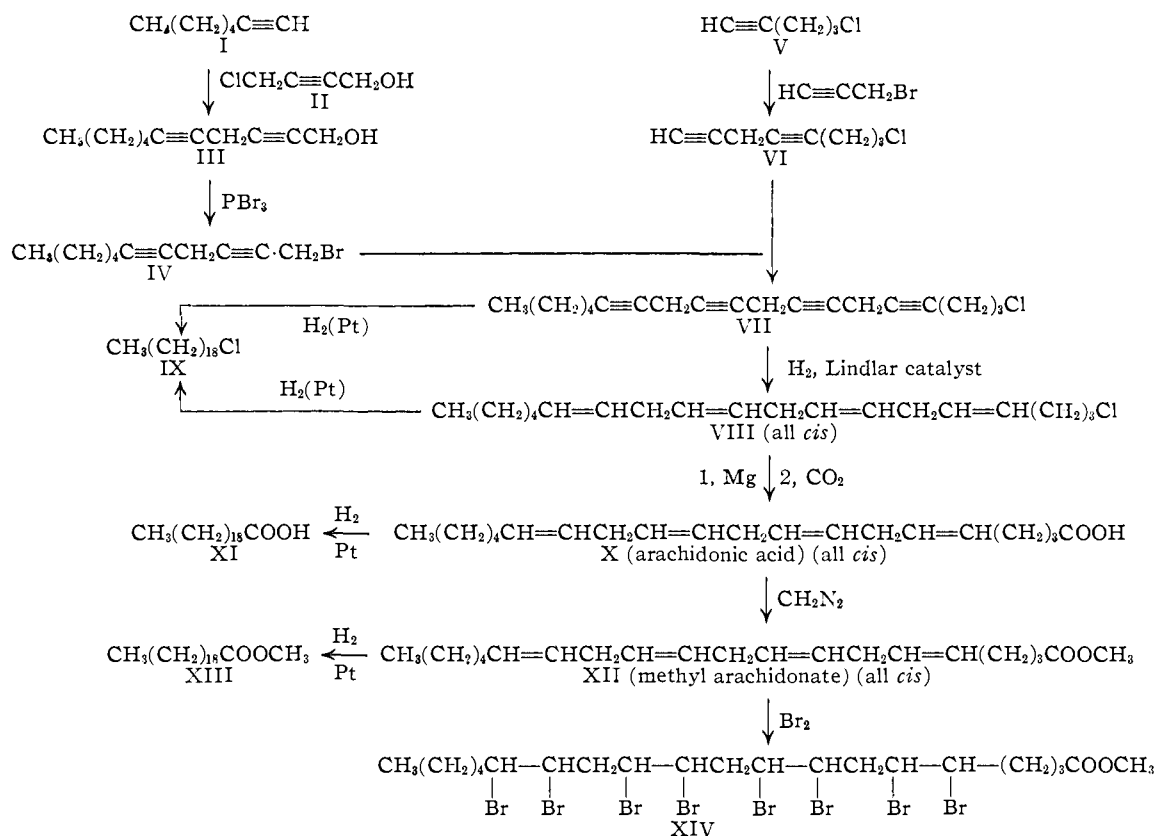 | | | | | | | |
      Br   Br   Br   Br   Br   Br   Br   Br
XIV

Fig. 1.

by chromatography gave the corresponding *cis*-tetraene VIII in 80% yield. The conditions of the hydrogenation had to be carefully controlled to minimize the formation of *trans*-bonded material. The chromatographic procedure using a somewhat higher proportion of activated alumina than is usual made it possible to separate relatively small amounts of hydrogenation product (probably a mixture) containing *trans* double bonds. Diisobutylaluminum hydride, which has been used before to reduce acetylenic and skipped diacetylenic compounds to the corresponding *cis*-ethylenes,[15] did not convert tetrayne VII to a pure product. Both tetrayne VII and tetraene VIII yielded nonadecyl chloride (IX) upon hydrogenation over platinum. Application of the entrainment method converted 1-chloro-4,7,10,13-nonadeca-tetraene (VIII) to its Grignard derivative, which reacted smoothly with carbon dioxide to give arachidonic acid (X). The acid could be hydrogenated to arachidic acid (XI) and could be esterified to methyl arachidonate (XII) (52% yield of ester from chloride VIII).

Methyl arachidonate, purified by chromatography over activated alumina, showed an infrared spectrum identical with that of natural methyl arachidonate except for a slightly stronger absorption at 966 cm.$^{-1}$. Quantitative determination[16]

making use of the absorption at this frequency revealed a maximum of 1.6–3.5% of ester with *trans* double bonds. Examination of the ester by ultraviolet spectroscopy showed only a low absorption, (ϵ 600–700) at 233 mμ, indicating 2–3% conjugated material. Full hydrogenation of the ester yielded methyl arachidate (XIII) identified by mixed melting point and infrared comparison with the saturated ester obtained from natural methyl arachidonate. The octabromide XIV of the synthetic ester gave no depression in melting point on admixture with a sample prepared from the natural ester. In gas–liquid chromatography, the same retention times were observed for the synthetic methyl arachidonate as for the ester from natural sources. On the basis of the above-mentioned comparisons with natural methyl arachidonate, we believe our synthetic material to be all-*cis*-methyl 5,8,11,14-eicosatetraenoate.

Acetylenic compounds III, IV, VI and VII show an intense infrared absorption band at 1308–1318 cm.$^{-1}$. We found this absorption—which has been attributed to the vibration of a methylene group attached to an acetylenic carbon atom[17]— to be more useful in indicating the presence of an internal acetylene than the weaker absorption in the 2230–2300 cm.$^{-1}$ region (C$\equiv$C stretching). Also noteworthy is the presence of a 970 cm.$^{-1}$

(14) H. Lindlar, *Helv. Chim. Acta*, **35**, 446 (1952).

(15) G. Wilke and H. Müller, *Chem. Ber.*, **89**, 444 (1956); *Ann.*, **629**, 222 (1960); K. Ziegler, H. G. Gellert, H. Lehmkuhl, W. Pfohl and K. Zosel, *ibid.*, **629**, 1 (1960); J. J. Bruno, unpublished work.

(16) Report of the Spectroscopy Committee, 1958–1959, *J. Am. Oil Chemists' Soc.*, **36**, 627 (1959). We wish to thank Dr. R. T. O'Connor,

Chairman, Spectroscopy Committee, A. O. C. S., for helpful correspondence and for providing us with standard comparison samples.

(17) J. H. Wotiz, Symposium on "Acetylene and its Derivatives," Am. Chem. Soc. 138th National Meeting, New York City, September 15, 1960; J. H. Wotiz, R. F. Adams, and C. G. Parsons, *J. Am. Chem. Soc.*, **83**, 373 (1961).

absorption peak, one ordinarily associated with the *trans* CH==CH grouping, in the infrared spectrum of all *cis*-tetraene chloride VIII. We suggest for the following reasons that this peak is attributable not to the presence of *trans* double-bonded impurities but rather to vibrational modes of a part of the molecule itself. First, careful rechromatography of tetraene chloride VIII over activated alumina failed to reduce the intensity of the 970 cm.$^{-1}$ band. Second, methyl arachidonate from pure VIII which had considerable absorption in the 970 cm.$^{-1}$ region, was shown to contain less than 3.5% *trans* double-bonded ester. When, on the other hand, methyl arachidonate was prepared from samples of the tetraene chloride VIII that showed greater absorption in this region than the purified material—and hence that undoubtedly contained *trans* double bonds—the infrared spectrum of the ester reflected the *trans* contamination of the precursor chloride by showing an intense absorption band at 965 cm.$^{-1}$, one corresponding to 30–50% *trans* impurities. Finally, a peak in this same region appears in the infrared absorption spectra of chlorodiyne VI and chlorotetrayne VII, neither of which have *trans* double bonds. Evidently structural features other than the *trans* double bond can result in absorption in this region.

Acknowledgment.—We wish to thank Drs. Aeschlimann and Rachlin of Hofmann–LaRoche, Inc., Nutley, N. J., for calling our attention to U. S. Patent 2,934,570,[10b] and for making a sample of synthetic methyl arachidonate available for comparison. Thanks are also due to Drs. Lipsky and Landowne for the gas–liquid chromatographic analyses which were performed at Yale University. Antara Chemicals, General Aniline and Film Corporation, New York, contributed generous quantities of 2-butyn-1,4-diol and propargyl bromide for which we are grateful.

Experimental

General Remarks.—All reactions were carried out in an atmosphere of pure dry nitrogen. In vacuum distillations nitrogen was bubbled through the capillary. All products were stored under vacuum in the refrigerator. The tetrahydrofuran (Fisher, reagent grade) was purified by treatment with potassium hydroxide pellets and dried by distillation from lithium aluminum hydride. Melting points were taken on a Fisher–Johns apparatus. Temperatures are not corrected. Infrared absorption curves were determined on a Perkin–Elmer double beam spectrophotometer (model 21). Ultraviolet absorption curves were taken with a Beckman DK recording spectrophotometer.

Coupling of Propargyl Bromide with 1-Heptyne. A. In Ether at 37°.—1-Heptyne (Farchan, n^{25}D 1.4057, 15.8 g., 0.16 mole) was converted to its Grignard derivative with ethylmagnesium bromide prepared from 4.0 g. (0.16 g. atom) of magnesium and 18.9 g. (0.17 mole) of ethyl bromide in 250 ml. of dry ether. Cuprous chloride, 0.5 g., and propargyl bromide (Antara, redistilled, 23.8 g., 0.20 mole) were added. The Grignard content of the reaction mixture was determined by titration.[18] The mixture was then refluxed and the temperature in the flask recorded as 37°. At intervals aliquots were withdrawn from the cooled reaction flask and titrated. At the end of 20.5 hours of reflux, it was found that 38% of the Grignard reagent was consumed. It took 115 hours for 96% of the reagent to be

used up. The reaction mixture was worked up by pouring it into a saturated ammonium chloride solution and extracting the aqueous layer with additional ether. The combined organic layers were washed with water and dried over magnesium sulfate. After filtration of the drying agent, the solvent was distilled off on a water-bath, and the residue fractionated through a 9-inch Vigreux column. The main fractions obtained were 8.9 g. (42%) of 1,4-decadiyne,[19] b.p. 60–62° (5 mm.), n^{25}D 1.4526, and 1.4 g. of a higher boiling fraction, probably 1,4,7-tridecatriyne, b.p. 79° (0.5 mm.). This corresponds to 8% yield of the triyne assuming that all of the propargyl bromide is available for triyne formation. 1,4-Decadiyne (neat) has absorption bands at 3280(s) (terminal acetylene), 2285(w) and 2230(w) (internal skipped acetylene), 2130(w) (terminal acetylene), 1310(s) (methylene–acetylene), and 972(w) cm.$^{-1}$. In addition, this sample showed a weak band at 1940 cm.$^{-1}$ indicating the presence of some allenic impurities.

B. In Tetrahydrofuran at 37°.—The coupling of 1-heptyne with propargyl bromide was run as described above except that tetrahydrofuran was used as the solvent. The reaction mixture was kept at 37° and the course of the reaction followed by titration.[18] At the end of 1 hour 87% of the Grignard reagent was consumed. After another 30 minutes only 4% Grignard reagent remained, and a thick yellow precipitate had been formed. The reaction was stopped at this stage and the product worked up. On distillation, 1,4-decadiyne, 9.2 g. (43%), b.p. 69–70° (9 mm.), n^{25}D 1.4517, was obtained along with only 0.5 g. (3%) of tridecatriyne, b.p. 80° (0.5 mm.). The decadiyne fraction had very little allene as shown by only a weak band at 1940 cm.$^{-1}$ in its infrared spectrum.

C. In Tetrahydrofuran at 65°.—The Grignard coupling reaction of 1-heptyne with propargyl bromide was repeated in tetrahydrofuran under reflux. The reaction mixture turned orange and a yellow precipitate appeared in 20 minutes after the addition of the propargyl bromide. Titration[18] at this point indicated 95% Grignard consumption. The usual work-up and distillation gave 4.3 g. (20%) of 1,4-decadiyne, b.p. 65–67° (10 mm.), n^{25}D 1.4539, and 4.0 g. (23%) of 1,4,7-tridecatriyne, b.p. 117° (4 mm.). The 1,4-decadiyne from this experiment exhibited the presence of more allene than the previous sample as evidenced by relatively greater absorption at 1940 cm.$^{-1}$ in the infrared.

Coupling of Propargyl Bromide with 5-Chloro-1-pentyne.—Preliminary experiments carried out on the coupling of propargyl bromide with the Grignard derivative of 5-chloro-1-pentyne gave results which were very similar to the ones described above. The detailed procedure for the coupling of these two compounds is described later as the synthesis of 8-chloro-1,4-octadiyne (VI).

A. In Ether at 37°.—In this solvent 90% (by titration[18]) of the Grignard reagent remained unchanged after 65 hours, and only a very small amount (*ca.* 1%) of 8-chloro-1,4-octadiyne, b.p. 60° (1 mm.), n^{25}D 1.4820, could be isolated. The infrared spectrum of the product showed the presence of a considerable amount of allenic impurities (band at 1940 cm.$^{-1}$).

B. In Tetrahydrofuran at 37°.—When tetrahydrofuran was used as solvent, 98% of the Grignard reagent was consumed in 1 hour. After work-up and distillation, 66% of 8-chloro-1,4-octadiyne, b.p. 61° (2 mm.), n^{25}D 1.4844, was obtained. A weak band at 1940 cm.$^{-1}$ in the infrared spectrum indicated the presence of a small amount of allene-containing material.

C. In Tetrahydrofuran at 65°.—At the reflux temperature of tetrahydrofuran, the Grignard reagent had disappeared in 90 minutes. In this case 40% of the chlorodiyne VI, b.p. 42–43° (0.2 mm.), n^{25}D 1.4851, was obtained. The allene content of this sample as evidenced by the infrared spectrum was greater than that of the sample obtained from the reaction carried out at 37°.

2,5-Undecadiyn-1-ol (III).—A Grignard reagent was prepared by boiling and magnetically stirring a mixture of 91 g. (0.84 mole) of dry ethyl bromide, 18 g. (0.74 g. atom) of magnesium and 600 ml. of tetrahydrofuran. A solution of 72 g. (0.75 mole) of 1-heptyne, in approximately 100 ml.

(18) H. Gilman, P. D. Wilkinson, W. P. Fischel and C. H. Meyers, *J. Am. Chem. Soc.*, **45**, 150 (1923). Best results were obtained by dissolving the aliquot in excess standard acid and back-titrating with standard base.

(19) 1,4-Decadiyne and 1,4,7-tridecatriyne were prepared in these laboratories by V. S. Gandhi (unpublished work) who reported b.p. 67–68° (10 mm.), n^{25}D 1.4503, for pure allene-free decadiyne, and b.p. 86° (0.3 mm.), n^{25}D 1.4780, for the triyne. Goldberg and Rachlin[10b] report for 1,4-decadiyne, b.p. 104° (50 mm.), $n^{24.5}$D 1.4515.

of tetrahydrofuran, was added rapidly to the clear solution of the Grignard reagent. The Grignard exchange occurred with vigorous boiling and gas evolution. After the addition of the 1-heptyne, the mixture was boiled and stirred for 45 minutes. Cuprous chloride (Merck, reagent grade, 1.0 g.) was added, and the boiling and stirring were continued for 15 minutes. 4-Chloro-2-butyn-1-ol, 31 g. (0.30 mole), b.p. 57–58° (0.5 mm.), n^{25}D 1.4987, prepared according to Bailey and Fujiwara[13] from 2-butyn-1,4-diol, was added rapidly in 75 ml. of tetrahydrofuran. During the subsequent 2 hours of stirring and boiling, the color of the mixture changed gradually from dark green-black to dark brown.

The coupling mixture was cooled (a light yellow precipitate formed) and poured into a liter of ice-water almost saturated with ammonium chloride. The aqueous layer was extracted with three 200-ml. portions of ether. The combined organic solutions were washed once with 100 ml. of almost saturated aqueous ammonium chloride and twice with 100-ml. portions of water. The solution was dried with magnesium sulfate, the solvents were removed by distillation at temperatures no higher than 70° at water-pump vacuum, and the residue was distilled through a 9-inch Vigreux column. The 42.2-g. fraction (87%) boiling at 86–91° (0.03 mm.) was taken as 2,5-undecadiyn-1-ol (III). This product, n^{25}D 1.4814, solidified in the refrigerator.[20] The compound, as a neat layer, had infrared absorption peaks at 3360(s) (hydroxyl); 2300(w), 2275(m) and 2230(w) (internal skipped acetylene); and 1315(s) cm.$^{-1}$ (methylene-acetylene).

Anal.[21] Calcd. for $C_{11}H_{16}O$: C, 80.4; H, 9.8. Found: C, 80.9; H, 9.9.

1-Bromo-2,5-undecadiyne (IV).—The bromo compound was prepared from 42.2 g. (0.26 mole) of 2,5-undecadiyn-1-ol (III), 31.4 g. (0.12 mole) of phosphorus tribromide in 75 ml. of ether (dried over sodium) and 5 ml. of pyridine (dried over sodium hydroxide pellets). The general directions of Taylor and Strong[22] were followed. The product, 1-bromo-2,5-undecadiyne (IV), b.p. 78–79° (0.01 mm.), n^{25}D 1.5061, was collected in 70% yield. Best results were obtained when the crude product in solution was washed only once with water. Excessive handling resulted in the appearance of hydroxyl and carbonyl peaks in the infrared absorption curves. The pure neat material absorbed in the infrared at 2298sh(w), 2275(m) and 2240(m) (internal skipped acetylene); and 1315(s) cm.$^{-1}$ (methylene-acetylene).

Anal. Calcd. for $C_{11}H_{15}Br$: C, 58.2; H, 6.7; Br, 35.2. Found: C, 58.0; H, 6.7; Br, 35.0.

8-Chloro-1,4-octadiyne (VI).—The Grignard reagent from 52.4 g. (0.48 mole) of ethyl bromide and 11.9 g. (0.49 g. atom) of magnesium in 400 ml. of tetrahydrofuran was allowed to react with 49.2 g. (0.48 mole) of 5-chloro-1-pentyne (Farchan, n^{25}D 1.4412) in 50 ml. of tetrahydrofuran in the manner described above for the preparation of 2,5-undecadiyn-1-ol (III). Cuprous chloride, 2.0 g., was added to the solution cooled to 15°, and stirring was continued for 15 minutes. With the temperature held at 20–30°, propargyl bromide (57.2 g., 0.48 mole) in 100 ml. of tetrahydrofuran was added rapidly. Cooling was necessary thereafter for about 30 minutes in order to keep the temperature below 30°. The yellow reaction mixture containing a precipitate was stirred for another hour, and was then poured over approximately 500 g. of ice and water almost saturated with ammonium chloride. The lower layer was extracted with ether, and the combined organic layers were washed with water and dried over magnesium sulfate. After removal of solvent at moderate temperatures under water-pump vacuum, distillation afforded 43.2 g. (64%) of 8-chloro-1,4-octadiyne (VI), b.p. 68–69° (2 mm.). As shown by the peak at 1940 cm.$^{-1}$ in the infrared absorption curve, some allenic material was present. However, boiling the product for 3 hours under nitrogen in an oil-bath at 170–180°[23] followed by redistillation in a small

flask provided with a 2-inch vacuum jacketed Vigreux column gave allene-free material, 35.8 g. (83% recovery), b.p. 61° (2 mm.), n^{25}D 1.4820. The neat product showed infrared absorption peaks at 3280(s) (terminal acetylene); 2285(w) and 2230(w) (internal skipped acetylene); 2130-(m) (terminal acetylene); 1308(s) (methylene-acetylene); and 972(m-s) cm.$^{-1}$.

Anal. Calcd. for C_8H_9Cl: C, 68.3; H, 6.5; Cl, 25.2. Found: C, 68.4; H, 6.4; Cl, 25.0.

1-Chloro-4,7,10,13-nonadecatetrayne (VII).—Ethyl bromide (5.4 g., 0.05 mole) was allowed to react with 1.2 g. (0.05 g. atom) of magnesium in 50 ml. of boiling tetrahydrofuran. The last small fragments of magnesium were destroyed by adding small additional quantities of ethyl bromide. The yields of coupling product were about the same when turnings from a bar of Dow resublimed magnesium (so that the solution of Grignard reagent was clear and colorless at this point) or when ordinary magnesium (Baker, purified) was used (so that the solution was pale brown). Freshly distilled 8-chloro-1,4-octadiyne (7.0 g., 0.05 mole) dissolved in some tetrahydrofuran was added to the stirred reaction mixture at 20°. After 45 minutes at 20°, no further evolution of gas was noted. Cuprous chloride, 0.25 g., was added, and stirring was continued for 20 minutes. Freshly distilled 1-bromo-2,5-undecadiyne (11.4 g., 0.05 mole) in 15–20 ml. of tetrahydrofuran was introduced rapidly, with the temperature held at 10–20°. Stirring the mixture, in which the amount of yellow precipitate gradually increased, was continued at 20° for 2 hours.

This coupling mixture was poured over 200 g. of ice-water almost saturated with ammonium chloride. Special care was taken to have all solutions saturated with nitrogen and kept blanketed with nitrogen as much as possible. The aqueous layer was extracted with ether, and the combined organic layers were washed twice with water. The solution, dried with magnesium sulfate, was exposed at 50° to water-pump suction. The residual oil was transferred without delay to a small Claisen flask and distilled rapidly by immersing the flask in an oil-bath preheated to 170–180°. After removal of a small forerun boiling up to 50° at 0.001 mm., 9.35 g. (65%) of pale yellow 1-chloro-4,7,10,-13-nonadecatetrayne (VII), n^{25}D 1.5088, was collected at b.p. 137° (0.0001 mm.). The yield of the product was *ca.* 20% lower if the tetrayne was allowed to remain overnight in ether between the work-up and the distillation. The melting point, determined by slowly warming the product sealed under vacuum in a small tube, is 11–13°. The neat material absorbed at 2285(w), 2265(w) and 2230(w) (internal skipped acetylene); 1318(s) (methylene-acetylene); and 975(m-s) cm.$^{-1}$. No bands were observed at 3280 and 2130 cm.$^{-1}$ (terminal acetylene). A 3.4 × 10^{-4} M isoöctane solution showed a low absorption maximum at 265 mμ (ϵ *ca.* 400).

Anal. Calcd. for $C_{19}H_{23}Cl$: C, 79.6; H, 8.1; Cl, 12.4. Found (for material with n^{25}D 1.5082): C, 79.8; H, 8.2; Cl, 12.0.

The molar refractivity for tetrayne VII calculated from its density, d^{25}_4 0.9783, and its index of refraction, n^{25}D 1.5085 (average), is 87.5. The molar refractivity calculated from bond values[24] is 88.1.

1-Chloro-4,7,10,13-nonadecatetrayne (VII) in air darkens rapidly and polymerizes to a dark resin. The material sealed under vacuum, could be stored as a solid at temperatures below 0°.

Stirring 0.75 g. of freshly distilled 1-chloro-4,7,10,13-nonadecatetrayne (VII) and 0.15 g. of platinum oxide in 25 ml. of ethyl acetate (Baker, analysed) under an atmosphere of hydrogen saturated the triple bonds. After the catalyst and solvent were removed, the hydrogenation product in pentane was filtered through activated alumina. Crystallization from pentane gave white crystalline nonadecyl chloride, m.p. 31–32°.[25] A 3% mixture with potassium bromide gave a simple absorption curve with split peaks appearing in the region of 2900 and 1470 cm.$^{-1}$, and single peaks at 730 and 718 cm.$^{-1}$.

(20) The melting point reported[10a] is 1.2–1.5°.

(21) Analyses were performed by Carol K. Fitz, Needham Heights, Mass., Schwarzkopf Microanalytical Laboratories, Woodside, N. Y., and S. Nagy, Microanalytical Laboratories, Massachusetts Institute of Technology, Cambridge, Mass.

(22) W. R. Taylor and F. M. Strong, *J. Am. Chem. Soc.*, **72**, 4263 (1950).

(23) W. J. Gensler and J. Casella, Jr., *ibid.*, **80**, 1376 (1958).

(24) A. I. Vogel, W. T. Cresswell, G. H. Jeffery and J. Leicester, *J. Chem. Soc.*, 514 (1952).

(25) N. Turkiewicz, *Ber.*, **72**, 1060 (1939), reported nonadecyl chloride, b.p. 164–167° (0.3 mm.). This compound has not been reported previously as a pure, crystalline, analyzed material.

Anal. Calcd. for $C_{19}H_{39}Cl$: C, 75.32; H, 12.98; Cl, 11.70. Found: C, 75.46; H, 12.81; Cl, 11.37.

Tetrayne VII, prepared in another way in 30% yield, was reported with b.p. 135° (<0.001 mm.), $n^{25}D$ 1.5053, and λ_{max} 270 mμ (ϵ 724).[10b]

1-Chloro-4,7,10,13-nonadecatetraene (VIII).—A mixture of 550 ml. of hexane (Fisher), 3.26 g. of Lindlar catalyst[14] and 0.82 g. of synthetic quinoline (Eastman Kodak white label) was stirred magnetically at room temperature under hydrogen in a 1-l. flask until hydrogen absorption ceased. 1-Chloro-4,7,10,13-nonadecatrayne (3.26 g., 0.011 mole), freshly distilled (b.p. 151° at 0.001 mm. $n^{25}D$ 1.5082), was dissolved in 50 ml. of hexane and added to the mixture, and hydrogenation was resumed. The rate of hydrogen absorption, controlled by the rate of stirring, was maintained at 5–6 ml. per minute. When close to four molar proportions of hydrogen had been taken up, an abrupt drop in rate (to about $^1/_{10}$th the former value) was evident, and the hydrogenation was interrupted at this stage.

After removing catalyst, the solution was washed with several portions of 5% hydrochloric acid followed by several portions of water until the washings were neutral to litmus. The solution was dried over sodium sulfate, and hexane was removed by distillation under water-pump vacuum at temperatures no higher than 40°. The residual oil, in 20 ml. of distilled pentane, was placed on a column of 500 g. of activated alumina (Alcoa, F-20). Pentane was passed through the column and 100-ml. portions of eluate were collected. Solvent was removed completely from each fraction by boiling on a steam-bath and then exposing the concentrated solution at temperatures around 50° to reduced pressure. Judging from differences at several frequencies, including absorption in the 965 cm.$^{-1}$ region, *trans* double-bonded products tended to concentrate in the first 8 fractions. The products from fractions 9–19, all of which had substantially the same infrared absorption curves, were combined and taken as 1-chloro-4,7,10,13-nonadecatetraene, 2.67 g. (80%). A sample distilled for analysis (b.p. 104–106° at 0.0001 mm.) in the manner described for tetrayne VII had $n^{25}D$ 1.4852. A 1.05 × 10^{-4} M isoöctane solution showed a poorly defined maximum at 234 mμ (ϵ ca. 900). Infrared absorption peaks for the neat liquid were noted at 1655(m) (double bond), 1445(s), 1395(s), 1380(m), 1345(w), 1325(w), 1308(m), 1285(m), 1270(s), 1043(m), 1015sh(w), 967(m), 915(m) and 730–670(s, unresolved) cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{31}Cl$: C, 77.38; H, 10.60; Cl, 12.02; double bonds, 4. Found: C, 77.16; H, 10.66; Cl, 12.20; double bonds by quantitative microhydrogenation, 3.9.

Gas–liquid chromatographic analysis by Dr. R. A. Landowne in the system described below for methyl arachidonate revealed no impurities. The observed retention time for the single peak was 21.5 min. When samples emerging in the earlier fractions of the alumina chromatography and showing greater infrared absorption at 967 cm.$^{-1}$ were analyzed by gas–liquid chromatography, two peaks appeared with retention times of 21.0 and 16.5 min. A neat layer of the faster-running *trans*-rich material, shows infrared absorption bands at 1642(w-m) (double bond), 1455 (s), 1403(w), 1378(m), 1345(w), 1307(m), 1285(m), 1270(m), 1040(w), 967(s) (in part, *trans* double bond) and 910(m) cm.$^{-1}$.

Half-hydrogenation conditions that gave too great a rate of hydrogen absorption led to increasing proportions of *trans* double-bonded products. The same was true when the amount of quinoline (or the activity of the catalyst) differed significantly from that given above. Temperature was also a factor.

Nonadecyl chloride (IX) was obtained by hydrogenation of 0.80 g. of tetraene VIII in ethyl acetate over platinum oxide. This nonadecyl chloride, as well as a mixture with the nonadecyl chloride from tetrayne VII, melted at 31–32°.[25] The infrared absorption curves of the two samples were the same.

Tetraene VIII has been prepared elsewhere in 69% yield. The constants reported are b.p. 96–98° (<0.001 mm.) and $n^{20}D$ 1.4871. The isopropyl alcohol solution showed an absorption shoulder at 225–230 mμ (ϵ 380) and an inflection at 260–270 mμ (ϵ 100).[10b]

Arachidonic Acid (X).—Ethyl bromide (redistilled, 2.0 g., 0.018 mole) in 40 ml. of tetrahydrofuran was allowed to

react with 1.2 g. (0.050 g. atom) of magnesium in the presence of a few crystals of iodine and mercuric chloride. The mixture was boiled and magnetically stirred for 1 hour under nitrogen in a 500-ml. round-bottom flask fitted with a condenser and dropping funnel. A solution of 2.67 g. (0.0091 mole) of 1-chloro-4,7,10,13-nonadecatetraene (VIII) and 0.4 g. (0.0037 mole) of ethyl bromide in 40 ml. of tetrahydrofuran was added to the boiling mixture over a period of 25 minutes. Boiling and stirring were continued for 15 hours, after which period, residual magnesium was destroyed by adding 3 g. of ethyl bromide in 35 ml. of tetrahydrofuran and boiling the mixture further for 30 minutes. A clear solution resulted.

A stream of carbon dioxide (generated from commercial Dry Ice) was introduced over the surface of the solution which was stirred and cooled to maintain an inside temperature of 15–25°. When there was no further tendency for the temperature to rise, the mixture was boiled in an atmosphere of carbon dioxide for 30 minutes. The carbonation mixture, cooled in an ice-bath, was decomposed with 250 ml. of 5% hydrochloric acid. The organic layer was washed with water until the washings were neutral to congo red and then dried with sodium sulfate.

Solvent and most of the propionic acid were removed by exposing the solution on the steam-bath to water-pump vacuum. An 8 × 10^{-5} M isoöctane solution of the residual arachidonic acid showed an absorption shoulder at 230–240 mμ (ϵ ca. 1000). The infrared spectrum of this synthetic arachidonic acid and that of natural arachidonic acid (Nutritional Biochemicals Corp., 90–95%), $n^{25}D$ 1.4888, were very similar.

Arachidic acid (XI) was obtained by hydrogenating 1.0 g. of the synthetic arachidonic acid in 40 ml. of ethyl acetate over 0.25 g. of platinum oxide to saturation. Two crystallizations of the hydrogenation product gave pure arachidic acid, m.p. 75.5–76.5°.[26] The same procedure with natural arachidonic acid gave arachidic acid (XI), which melted before and after mixing with synthetic arachidic acid at 75.5–76.5°. Different crystalline forms of arachidic acid were observed, which had different infrared absorption spectra in potassium bromide pellets, but identical infrared absorption spectra in chloroform solution. One form was obtained as tiny crystals by cooling an ether solution; another form was obtained as large tetrahedral crystals when the ether solution evaporated slowly.

Methyl Arachidonate (XII).—A stirred ethereal solution of arachidonic acid from 2.67 g. of tetraene VIII in 100 ml. of ether was treated in the ice-bath with an ether solution of diazomethane (excess) prepared from 10 g. of N-nitroso-N-methyl-p-toluenesulfonamide.[27] After the yellow solution had been stirred at room temperature for 30 minutes, solvent and excess diazomethane were removed by vacuum distillation at temperatures up to 40°. The pale yellow residual oil dissolved in 20 ml. of redistilled pentane was placed on a column of activated alumina (500 g., Alcoa, F-20). Elution first with pentane (total volume 800 ml.) and then with pentane containing 5% ether furnished a series of 100-ml. fractions. Each fraction was stripped of solvent and finally held under oil-pump vacuum at 40° for 15 minutes. The infrared absorption curves of the residues were then examined. Some unchanged 1-chloro-4,7,10,13-nonadecatetraene (VIII) appeared in fractions 5–8. Combined fractions 19–33 furnished 1.5 g. (52%) of oil, which on distillation in a 5-ml. Claisen flask deeply immersed in an oil-bath gave water-white methyl arachidonate (XII), b.p. 127–128° (0.001 mm.), $n^{25}D$ 1.4775, with almost no loss of material. Other index of refraction values reported for methyl arachidonate are $n^{20}D$ 1.4799[28] and $n^{20}D$ 1.4806[10b]. We noted for a sample of methyl arachidonate (Hormel, derived from pork liver fat), $n^{25}D$ 1.4778.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 78.89; H, 10.67.

The ultraviolet absorption curve taken with a 2.5 × 10^{-4} M isoöctane solution revealed an ill-defined maximum, almost a shoulder, at 233 mμ (ϵ ca. 700). On the assumption that ϵ is 26,000 to 33,300[29,30] for the conjugated diene system,

(26) F. Francis and S. H. Piper, *J. Am. Chem. Soc.*, **61**, 577 (1939), reported m.p. 75.35°.

(27) T. J. DeBoer and H. J. Backer, *Rec. trav. chim.*, **73**, 229 (1954).

(28) O. S. Privett, R. P. Weber and E. C. Nickell, *J. Am. Oil Chemists' Soc.*, **36**, 443 (1959).

(29) C. R. Smith, T. L. Wilson, E. H. Melvin and I. A. Wolff,

the synthetic ester contained *ca.* 2–3% of isomeric conjugated material. The infrared absorption curve of the neat synthetic ester XII was identical with that of purified methyl arachidonate from natural sources (Hormel). We have also compared the infrared curves of our synthetic methyl arachidonate with a sample of ester prepared at Hofmann–La-Roche[10b] (n^{25}D 1.4778) and found them to be the same. Quantitative determination of *trans* double-bonded material in the synthetic ester XII according to a standard infrared procedure[16] revealed 3.5 % of *trans* double-bonded material. This sample of synthetic methyl arachidonate was analyzed on a Barber–Colman instrument (ionization detector) by Dr. R. A. Landowne at Yale University School of Medicine. He used an 8-ft. column (1/4″, glass) packed with 14.4% of polyethylene glycol succinate on 100–140 mesh Chromosorb P at 167° and with 40 pounds per square inch of argon (inlet pressure). Under these conditions both synthetic and natural esters showed a retention time of 57 min. (within 1 minute). This sample of synthetic ester had an impurity of about 9% which showed up as an additional peak with a retention time of 49 min.

Other preparations of methyl arachidonate were carried out using the procedure described above. The product in these cases was purified by chromatography over activated alumina. Distillation was avoided as it was suspected of giving rise to isomerization in methyl arachidonate.[28] Samples of synthetic ester prepared in this way were found to contain less than 2% of *trans* double-bonded material as judged by their absorption at 966 cm.$^{-1}$ in the infrared, and *ca.* 2% of conjugated material as judged from their ultraviolet spectra.

For instance, when arachidonic acid from 1.0 g. of tetra-

J. Am. Chem. Soc., **82**, 1417 (1960); S. Sparreboom, *Konikl. Ned. Akad. Wetenschap.*, *Proc. Ser. B*, **59**, 472 (1956) [*C. A.*, **51**, 11992 (1957)]; P. L. Nichols, Jr., S. F. Herb and R. W. Riemenschneider, *J. Am. Chem. Soc.*, **73**, 247 (1951).

(30) R. R. Allen, *J. Org. Chem.*, **21**, 143 (1956); F. D. Gunstone and W. C. Russell, *J. Chem. Soc.*, 3782 (1955).

ene chloride VIII was esterified with diazomethane and chromatographed over *ca.* 200 g. of activated alumina as described above, the ester obtained was found by standard infrared procedures[16] to contain 1.6% *trans* double-bonded material. The infrared curve of the neat ester was identical with that of the ester from natural sources (Hormel). Its ultraviolet spectrum, taken with a 2.1 × 10^{-4} *M* isoöctane solution, had a shoulder at 233 mμ with ϵ *ca.* 600. This sample of ester XII was analyzed by gas–liquid chromatography on an Aerograph instrument (hot filament detector) with an LAC 446 column (5 ft., 1/4″ o.d., stainless steel) at 209° and a flow rate of 160 ml. per minute of helium. Under these conditions, a single peak with a retention time of 38 min. was observed for the synthetic ester. For comparison, the same retention time was observed when natural methyl arachidonate was run under identical conditions.

Methyl arachidate (XIII) was prepared by stirring 0.8 g. of methyl arachidonate with 30 ml. of ethyl acetate and 0.15 g. of platinum oxide under hydrogen until hydrogen absorption came to a stop. The product in pentane was filtered through 2 g. of activated alumina and was recrystallized from the same solvent. Methyl arachidate from synthetic methyl arachidonate melted sharply at 46.5°. Methyl arachidate from natural methyl arachidonate melted at 45.5° A mixture melted at 46°. The infrared absorption curves of the two saturated esters in potassium bromide pellets were identical.

The octabromide (XIV) of methyl arachidonate was prepared by adding bromine by drops to 0.2 g. of the ester in 20 ml. of ether cooled in an ice-bath until the orange color persisted. The solids formed on keeping the ether solution in the refrigerator overnight were collected and washed with cold ether and then dried. The octabromides derived from synthetic and from natural methyl arachidonate as well as a mixture of the two octabromides melted at 232–233°.[31]

(31) G. Y. Shinowara and J. B. Brown, *J. Biol. Chem.*, **134**, 331 (1940); report m.p. 228.5–229.5°; Osbond and Wickens[10a] report m.p. 228–229°.

The Structure of Streptimidone

BY PETER W. K. WOO, HENRY W. DION AND QUENTIN R. BARTZ

RECEIVED FEBRUARY 10, 1961

The structure of streptimidone has been confirmed by chemical evidence as 3-(2-hydroxy-5,7-dimethyl-4-oxo-6,8-nonadienyl)-glutarimide.

The chemistry of streptimidone (C$_{16}$H$_{23}$NO$_4$) was reported recently,[1] and Ia has been assigned as its structure.[2]

CH$_3$　　CH$_3$　O

CH$_2$=CHC=CHCH—C—CH$_2$R

9　　8　7　6　5　　4　3

Ia,b

CH$_3$　　CH$_3$　O

CH$_3$CH$_2$CHCH$_2$CH—C—CH$_2$R

IIa,b

　　　OH　　　　CH$_2$—CO

a, R = -CHCH$_2$CH　　　　　NH

　　　2　1　　　CH$_2$—CO

b, R = H

The β-hydroxyketone system in streptimidone (Ia) and tetrahydrostreptimidone (IIa) easily undergoes the reverse aldol reaction in alkali to give (a) aldehyde derivatives from the β-ethylglu-

(1) R. P. Frohardt, H. W. Dion, Z. L. Jakubowski, A. Ryder, J. C. French and Q. R. Bartz, *J. Am. Chem. Soc.*, **81**, 5500 (1959).

(2) E. E. van Tamelen and V. Haarstad, *ibid.*, **82**, 2974 (1960).

tarimide moiety, the structure of which has been firmly established,[1] and (b) the corresponding C-9 methyl ketones (Ib, IIb). Available data supporting the formulation of the C-9 moiety as in I, which is a modification of the original proposal of Frohardt, *et al.*,[1] may be summarized as: ultraviolet and infrared data indicating the presence of a conjugated diene unit,[3] facile base-catalyzed conversion of the existing ultraviolet chromophore to a 2,4-dienone system, and the n.m.r. spectrum of streptimidone acetate[2] showing five hydrogens (four olefinic and one acetoxyl methine) in the olefinic hydrogen region and a doublet for the C-methyl group. The present investigation, which was undertaken to provide definitive chemical data, has confirmed this formulation by (a) unequivocally establishing the carbon skeleton of the C-9 moiety through a degradation series, and (b) locating the diene unit on this skeleton by careful

(3) The conjugated diene unit in streptimidone is shown by: (a) the ultraviolet spectrum, λ_{max}^{MeOH} 232 mμ, ϵ 23,100; (b) two infrared absorption bands at 6.09(m) and 6.21(w) μ; (c) the ultraviolet spectrum of the sodium borohydride reduction product, λ_{max}^{MeOH} 230 mμ, ϵ 23,600.[1]