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 tetraene 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]

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).

(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°.

[CONTRIBUTION FROM THE RESEARCH DIVISION, PARKE, DAVIS & CO., DETROIT 32, MICH.]

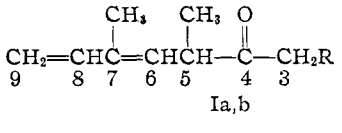
The Structure of Streptimidone

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

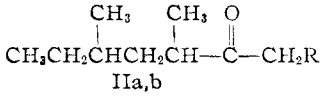
RECEIVED FEBRUARY 10, 1961

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

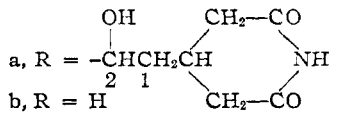
The chemistry of streptimidone ($C_{16}H_{23}NO_4$) was reported recently,[1] and Ia has been assigned as its structure.[2]



Ia,b



IIa,b



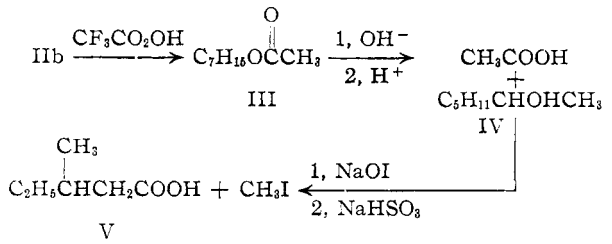
The β-hydroxyketone system in streptimidone (Ia) and tetrahydrostreptimidone (IIa) easily undergoes the reverse aldol reaction in alkali to give (a) aldehyde derivatives from the β-ethylglutarimide moiety, the structure of which has been firmly established,[1] and (b) the corresponding C-9 methyl ketones (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

(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).

(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]

reinvestigation of the previously reported ozonolysis experiments.[1]



The C-9 ketone from tetrahydrostreptimidone[1] was shown to be 3,5-dimethyl-2-heptanone (IIb) by the following reactions. Oxidation of this ketone with trifluoroperacetic acid[4] gave III (2-acetoxy-4-methylhexane). Alkaline hydrolysis of III yielded acetic acid, isolated in 75% yield, and the C-7 alcohol IV (4-methyl-2-hexanol). The structure of IV was proved by sodium hypoiodite oxidation, which yielded iodoform (indicative of a methylcarbinol group in IV and hence the methyl group at C-5 of streptimidone) and 3-methylvaleric acid (V), identified as its *p*-toluide. Chromium trioxide oxidation of the alcohol IV yielded the corresponding ketone isolated as the 2,4-dinitrophenylhydrazone.

Ozonolysis of streptimidone (Ia) yielded a mixture of steam-volatile carbonyl compounds, which were identified as the individual pure 2,4-dinitrophenylhydrazone derivatives. The mixture was thus found to consist of formaldehyde (20.6% yield of the 2,4-dinitrophenylhydrazone based on streptimidone), pyruvaldehyde (5.2%) and acetaldehyde (1.4%). In contrast with the earlier experiments,[1] no methyl ethyl ketone was detected. It was found that the reagent grade ethyl acetate (further distilled from 2,4-dinitrophenylhydrazine) used as a solvent in the isolation procedure of some of these earlier experiments contained methyl ethyl ketone at approximately 40 μg./ml. The detection of formaldehyde and pyruvaldehyde requires the placement of the conjugated diene system[3] in streptimidone as indicated in Ia, thus confirming the elegant assignment made earlier by van Tamelen and Haarstad from recorded properties[1] and n.m.r. data.[2]

Experimental

Alkaline hydrolysis of tetrahydrostreptimidone (IIa) was performed according to the reported procedure.[1] A solution of 25.1 g. (84.4 mmoles) of tetrahydrostreptimidone in 190 ml. of 1.25 *N* sodium hydroxide was steam distilled for 1.5 hours. The distillate, 750 ml., was extracted three times with 200-ml. portions of ether. After drying with anhydrous sodium sulfate, the ether extract was distilled to yield 9.5 g. (79%) of the C-9 volatile oil IIb, b.p. 174–175°.

Degradation of the C-9 Oil (IIb). A. Oxidation of IIb with Trifluoroperacetic Acid.—Trifluoroacetic anhydride (21 ml., 148 mmoles) was added dropwise to a stirred solution of 3.39 ml. (125 mmoles) of 90% hydrogen peroxide in 20 ml. of ice-cold methylene chloride. The resulting solution was added during a 30-minute period to a stirred suspension containing 53 g. (373 mmoles) of dry, finely ground disodium hydrogen phosphate, 9.5 g. (67 mmoles) of the C-9 ketone IIb and 70 ml. of methylene chloride. The mixture, which boiled vigorously during the addition, was heated under reflux for an additional 40 minutes. The insoluble salts were filtered and washed with methylene chloride, and the combined filtrates were washed with 10% sodium bicarbonate solution, dried over magnesium sulfate and distilled. Two fractions of colorless distillate, acetate III, were collected: (a) 4.59 g. (43.3%), b.p. 162–167.5°; and (b) 4.42 g. (41.7%), b.p. 167.5–168.0°, $n^{28}D$ 1.4059. Since the two fractions exhibited identical infrared spectra, they were combined for subsequent degradation. Fraction b was analyzed.

Anal. Calcd. for $C_9H_{18}O_2$: C, 68.31; H, 11.47. Found: C, 68.16; H, 11.73.

B. Alkaline Hydrolysis of Acetate III.—A mixture of 8.8 g. (55.6 mmoles) of acetate III, 16 ml. of 6 *N* sodium hydroxide and 30 ml. of methanol was heated under reflux for 3 hours. The resulting solution was distilled until 23 ml. of distillate, containing mostly methanol (b.p. 62–68°), was removed. The C-7 alcohol IV, which formed an oily layer in the residue, was separated. The aqueous phase was saturated with sodium carbonate and extracted with ether. The extract was dried over magnesium sulfate, and the ether was distilled off, leaving additional C-7 alcohol. The two fractions of C-7 alcohol were combined, dried over magnesium sulfate and distilled to give 6.38 g. (98.6%) of distillate, b.p. 131–143°. Part of this was dried over anhydrous potassium carbonate for 3 days and distilled to give pure C-7 alcohol IV, b.p. 148°, $n^{28}D$ 1.4183, $[\alpha]^{25}D$ 0 (*c* 2.40%, methanol).

Anal. Calcd. for $C_7H_{16}O$: C, 72.35; H, 13.88. Found: C, 72.35; H, 13.98.

In a different hydrolytic run employing 533 mg. (3.37 mmoles) of acetate III, the hydrolysate, after concentration and ether extraction according to the above procedure (omitting the use of sodium carbonate for salting out), was acidified with sulfuric acid and distilled. The distillate was titrated with sodium hydroxide (2.54 meq., 75.4%) and then lyophilized to give 211 mg. (76.3%) of sodium acetate, identified by its infrared spectrum and further characterized as its *p*-bromophenacyl ester, m.p. 85.5–86.0°, which was shown to be completely identical with an authentic sample by mixed-melting point determination and comparison of infrared spectra.

C. Sodium Hypoiodite Oxidation of C-7 Alcohol IV.—To a mixture containing 2.35 g. (20.2 mmoles) of the C-7 alcohol IV, 50 ml. of dioxane and 57 ml. of 10% sodium hydroxide solution, was added with swirling 160 ml. of the oxidizing agent (18.5 g. of iodine, 37 g. of potassium iodide, 148 ml. of water) at such a rate that a slight excess of iodine was maintained throughout. The iodoform, which precipitated, weighed 2.19 g. (27.5%), m.p. 119–121° dec. Extraction of the solution with ether yielded an additional 320 mg. (4.0%) of iodoform.

To the extracted solution was added 22 ml. of a 20% sodium bisulfite solution. Extraction of the resulting acidic solution with ether, desiccation with magnesium sulfate and distillation yielded 652 mg. of a dark brown distillate, b.p. 110–190°, which showed a retention time identical with that of 3-methylvaleric acid in vapor phase chromatography. A solution of the distillate in *n*-pentane was washed with aqueous sodium bisulfite, then water, and finally dried with magnesium sulfate and distilled to give fraction A, b.p. 110–180°, 428 mg., light brown, and fraction B, 180–190°, 148 mg., dark brown.

Part of fraction A, 150 mg., was converted to the *p*-toluide *via* the acid chloride[5] by successive treatment with thionyl chloride and *p*-toluidine. The product, 130 mg. of crystals from methanol–water, m.p. 72.5–73.5°, was found to be completely identical with an authentic sample of 3-methylvaleryl-*p*-toluide by mixed melting point determination and comparison of infrared spectra.

Anal. Calcd. for $C_{13}H_{19}NO$: C, 76.05; H, 9.33; N, 6.82. Found: C, 75.92; H, 9.23; N, 6.77.

D. Chromium Trioxide Oxidation of the C-7 Alcohol IV.—To a cold solution of 59.5 mg. (0.511 mmole) of the C-7 alcohol IV in 0.25 ml. of glacial acetic acid was added dropwise a solution of 34.8 mg. (0.348 mmole) of chromium trioxide in 0.25 ml. of 10 *N* acetic acid. After 1 day at room temperature, the reaction mixture was adjusted to *p*H 5 with dilute sodium hydroxide and extracted with ether. After evaporation of the ether, the residual ketone

(4) W. D. Emmons and G. B. Lucas, *J. Am. Chem. Soc.*, **77**, 2287 (1955).

(5) N. D. Cheronis, "Micro and Semimicro Methods," in "Technique of Organic Chemistry," Vol. VI, ed. by A. Weissberger, Interscience Publ., Inc., New York, N. Y., 1954, p. 538.

was treated with 204 mg. of 2,4-dinitrophenylhydrazine in 30 ml. of 2 *N* hydrochloric acid and 10 ml. of methanol. The resulting 2,4-dinitrophenylhydrazone was removed by extraction with benzene and purified by chromatography through a deactivated alumina column.[6] Further purification was achieved by chromatography on a silicic acid–Celite (2:1) column.[7] The main fraction, eluted with 5% ether in petroleum ether, yielded 60 mg. of product, which was recrystallized three times from *n*-heptane to give 6.8 mg. of orange crystals, m.p. 45–48°.[8] An additional 16 mg. of crystals, m.p. 45–51°, was obtained similarly from the mother liquor.

Anal. Calcd. for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04. Found: C, 53.37; H, 6.42; N, 18.87.

Ozonolysis of Streptimidone (Ia).—Ozone was passed through a solution of 866 mg. (2.95 mmoles) of crystalline streptimidone in 20 ml. of ethyl acetate (distilled three times from 2,4-dinitrophenylhydrazine) at −80° for 45 minutes. The blue color of ozone appeared after 5 minutes of ozonization. The ethyl acetate solution was concentrated *in vacuo* to approximately 10 ml., then mixed with 10 ml. of methanol, 1 g. of ferrous sulfate and 175 ml. of water. The mixture was steam distilled (525 ml. of distillate) into a 2 *N* hydrochloric acid solution saturated with 2,4-dinitrophenylhydrazine (DNP mixture).

The precipitate from the DNP mixture was filtered, washed with methanol and dried to give 67 mg. (5.2%) of a rust colored precipitate, which was crystallized first from dimethylformamide–water and then from dimethylformamide to give 31 mg. (2.4%) of pyruvaldehyde bis-2,4-dinitrophenylhydrazone, identified by comparison with an authentic sample (no mixed melting point depression; identical ultraviolet and infrared spectra).

Anal. Calcd. for $C_{15}H_{12}N_8O_8$: C, 41.67; H, 2.80; N, 25.92. Found: C, 41.68; H, 2.70; N, 26.11.

The filtrate and washings from the DNP mixture were extracted with chloroform. The extract was evaporated to dryness *in vacuo*, and a benzene solution of the residue was percolated through a deactivated alumina[6] column to remove 2,4-dinitrophenylhydrazine. Evaporation of the effluent to dryness yielded 137 mg. of crystals, which was recrystallized from methanol to give 75 mg. (12.1%) of formaldehyde 2,4-dinitrophenylhydrazone, m.p. 158–163°, identified by its infrared spectrum and by paper chromatography, using *n*-heptane saturated with methanol.[9] Paper chromatography showed that the mother liquor from above contained an additional 53 mg. of formaldehyde 2,4-DNP (8.5%) and 9 mg. of acetaldehyde 2,4-DNP (1.4%).

As a control experiment, employing completely identical isolation procedures, ozonolysis of the C-9 ketone from dihydrostreptimidone (streptimidone with one olefinic linkage catalytically reduced[1]) yielded methyl ethyl ketone (39%) as the major product.

The reagent grade ethyl acetate used in the isolation procedures in some of the previously reported ozonolysis experiments[1] was shaken with 2 *N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine. The weight of the resulting methyl ethyl ketone 2,4-dinitrophenylhydrazone isolated indicated that the ethyl acetate contained methyl ethyl ketone at approximately 40 μg./ml. Thus, in the ozonolysis experiments of the present investigation, meticulous removal of any ketones and aldehydes from all solvents was performed, by repeated distillation from solid 2,4-dinitrophenylhydrazine.

Acknowledgment.—The authors wish to express their appreciation to Dr. H. E. Machamer and associates for supplying pilot plant quantities of culture filtrates and crude concentrates; to Dr. D. H. Szulczewski and associates for ultraviolet and infrared determinations; and to Mr. C. E. Childs and associates for microanalyses.

(6) Alcoa F-20 alumina was adjusted to *p*H 5 with dilute sulfuric acid and then dried for 4 hours at 200°.

(7) B. E. Gordon, F. Wopat, Jr., H. D. Burnham and L. C. Jones, Jr., *Anal. Chem.*, **23**, 1754 (1951).

(8) M. C. Chiang [*J. Chinese Chem. Soc.*, **18**, 65 (1951); *cf. C. A.*, **46**, 4472 (1952)] reported 45–47° as the melting range of 4-methyl-2-hexanone 2,4-dinitrophenylhydrazone.

(9) D. F. Meigh, *Nature*, **170**, 579 (1952).

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION, CIBA PHARMACEUTICAL PRODUCTS, INC., SUMMIT, N. J.]

Investigations in Heterocycles. X.[1] The Synthesis of Tetrahydro-1,3-benzodiazepines, a New Heterocyclic System

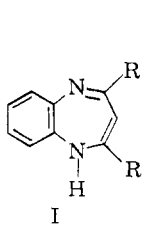
BY GEORGE DESTEVENS AND MARYLOU DUGHI

RECEIVED FEBRUARY 6, 1961

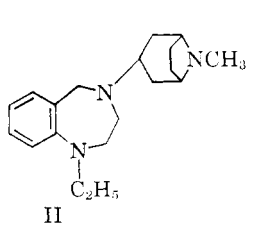
A method has been developed for the preparation of 3-substituted-1,2,4,5-tetrahydro-1,3-benzodiazepines. The structure of these compounds has been rigorously proved by means of chemical transformations and spectral data.

Within the past decade the synthesis of seven-membered ring compounds containing two nitrogen atoms has attracted considerable attention. Ried[2] and co-workers have enlarged significantly upon the early findings of Thiele[3] and Steimmig concerning the synthesis of 2,4-disubstituted-1H-1,5-benzodiazepines (I), whereas the preparation of 1-ethyl-4-(3-tropanyl)-tetrahydro-1H-1,4-benzodiazepine (II) and its derivatives has been the subject of a report by Archer, *et al.*[4]

Finally, another member of this group, 1-H-1,3-benzodiazepine, has recently been described only in derivative form by Plieninger[5] and Nogradi.



I



II

They have found that the autoxidation of the lactone β-(*o*-acetamidophenyl)-α-amino-γ-hydroxycrotonic acid (III) gives rise to the lactone of 5-hydroxymethyl-2-methyl-1H-1,3-benzodiazepine-4-carboxylic acid (IV).

Our interest in seven-membered heterocycles of the 1,3-benzodiazepine class was confined to the study of the completely saturated system, 1,2,4,5-tetrahydro-3-methyl-1,3-benzodiazepine (V) and its analogs.

(1) For part IX in this series see G. deStevens, A. Halamandaris, P. Wenk and L. Dorfman, *J. Am. Chem. Soc.*, **81**, 6292 (1959).

(2) W. Ried and A. Draisbach, *Chem. Ber.*, **92**, 949 (1959); W. Ried and E. Torinus, *ibid.*, **92**, 2902 (1959).

(3) J. Thiele and G. Steimmig, *ibid.*, **40**, 955 (1907).

(4) S. Archer, J. R. Lewis, M. J. Unser, J. O. Hoppe and H. Lape, *J. Am. Chem. Soc.*, **79**, 5783 (1957).

(5) H. Plieninger and I. Nogradi, *Ber.*, **88**, 1965 (1955).