

[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY¹]**Dimorphecolic Acid—A Unique Hydroxydienoid Fatty Acid²**

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Dimorphecolic acid, the chief constituent fatty acid of *Dimorphothea aurantiaca* seed oil, is characterized as an optically active 9-hydroxy-*trans,trans*-10,12-octadecadienoic acid.

Screening a considerable number of previously uninvestigated species revealed unusual properties of seed oils from several members of the family Compositae. These oils were of interest in connection with a search for new industrial oilseed crops. Earle, Jones and Wolff³ reported that the seed of *Dimorphothea aurantiaca* yielded an oil *ca.* 48% of whose acids contained *trans,trans*-conjugated diene. Comparable concentrations of epoxy and hydroxy acids were also indicated. This paper describes the isolation and proof of structure of a new fatty acid contained in that oil.

Saponification of oil obtained by petroleum ether extraction of *Dimorphothea aurantiaca* seed was not used when it was found that the conjugated dienoid system was inordinately sensitive to alkali under certain conditions. It was found, however, that methyl esters could be prepared by a transesterification procedure without destruction of the diene moiety. The esters were separated by countercurrent distribution, using the solvent system acetone-triethylhexane.⁴ After 30 transfers, the methyl ester (I) of a new fatty acid, for which the name *dimorphecolic acid* is proposed, was obtained as a distinct peak with its maximum at tube 5 (see Fig. 1). This liquid ester, which constituted about 65% of the total, was weakly dextrorotatory in chloroform. Quantitative hydrogenation indicated that it contained two double bonds. Its ultraviolet spectrum, characterized by a maximum at 231 μ , showed the double bonds to be conjugated. The infrared spectrum of this ester indicated that its conjugated system had the *trans,trans*-configuration; this assignment followed from the fact that there was a single peak in the 10–11 μ region, at 10.09 μ .⁵ One mole of hydrogen bromide was consumed by I in the Durbetaki hydrogen bromide-acetic acid titration for oxirane oxygen.⁶ Spectral evidence indicated the presence of a hydroxyl group, but no epoxy group. The products accompanying methyl dimorphecolate and concentrated in tubes 16–29 were apparently predominantly esters of fatty acids of

the common types. Of these, 11% were saturated, 45% were monoethenoid and 33% were non-conjugated diethenoid acids.⁷

To resolve the apparent conflict between infrared evidence and other data, methyl dimorphecolate (I) was refluxed for 1 hour in glacial acetic acid, following Gunstone's procedure for acetolysis of epoxy acids.⁸ Somewhat surprisingly, this treatment produced a *conjugated triene* (II) which contained *no* hydroxyl group. This reaction demonstrated the absence of an epoxy group in I and suggested that the hydroxyl group was located on an α -carbon with respect to the conjugated diene. Absence of epoxide groupings was further confirmed by lithium aluminum hydride reduction of I to the crystalline diene-diol VII, which consumed 1 mole of hydrogen bromide, apparently as rapidly as I. Confirmation for presence of the system $-\text{CH}=\text{CHCH}=\text{CHCHOH}-$ was obtained by oxidizing I to IV with chromium trioxide-pyridine; IV had an ultraviolet absorption maximum at 275 μ , in harmony with an α,β - and γ,δ -unsaturated ketone. Dehydrated product II was hydrogenated to saturated ester III, which was identified unequivocally as methyl stearate by mixed melting point determinations and by gas chromatography. A normal C-18 carbon skeleton for dimorphecolic acid was thereby established.

Direct hydrogenation of I yielded a crystalline saturated hydroxy ester V, shown by infrared spectra and melting point comparisons to be an optically active methyl 9-hydroxyoctadecanoate. Compound V was oxidized by chromium trioxide-acetic acid to VI, which was shown by infrared comparison to be identical with methyl 9-oxooctadecanoate.

The position of the hydroxyl in dimorphecolic acid having been settled, there remained the question of the position of the conjugated double bonds, which could be either in 5,7- or 10,12-positions. The issue was decided in favor of the 10,12-positions by ozonolysis. Ozonolysis of VII followed by reductive cleavage yielded aldehydes VIII and X, and presumably glyoxal IX as well; these were separated by solvent extraction and steam distillation and isolated as 2,4-dinitrophenylhydrazones. The derivative of VIII was rigorously identified as hexanal 2,4-dinitrophenylhydrazone by mixed melting point observations, by paper chromatography, and by X-ray diffraction.

Separation of the derivatives of the non-volatile aldehydes proved difficult, and it appeared that a rather complex mixture was obtained. One crystalline derivative obtained in small yield (XI) was

(1) This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) Presented before the Division of Organic Chemistry, 135th Meeting American Chemical Society, Boston, Mass., April 5–10, 1959.

(3) F. R. Earle, Q. Jones and I. A. Wolff, presented before the 32nd Fall Meeting, American Oil Chemists' Society, Chicago, Ill., October 20–22, 1958.

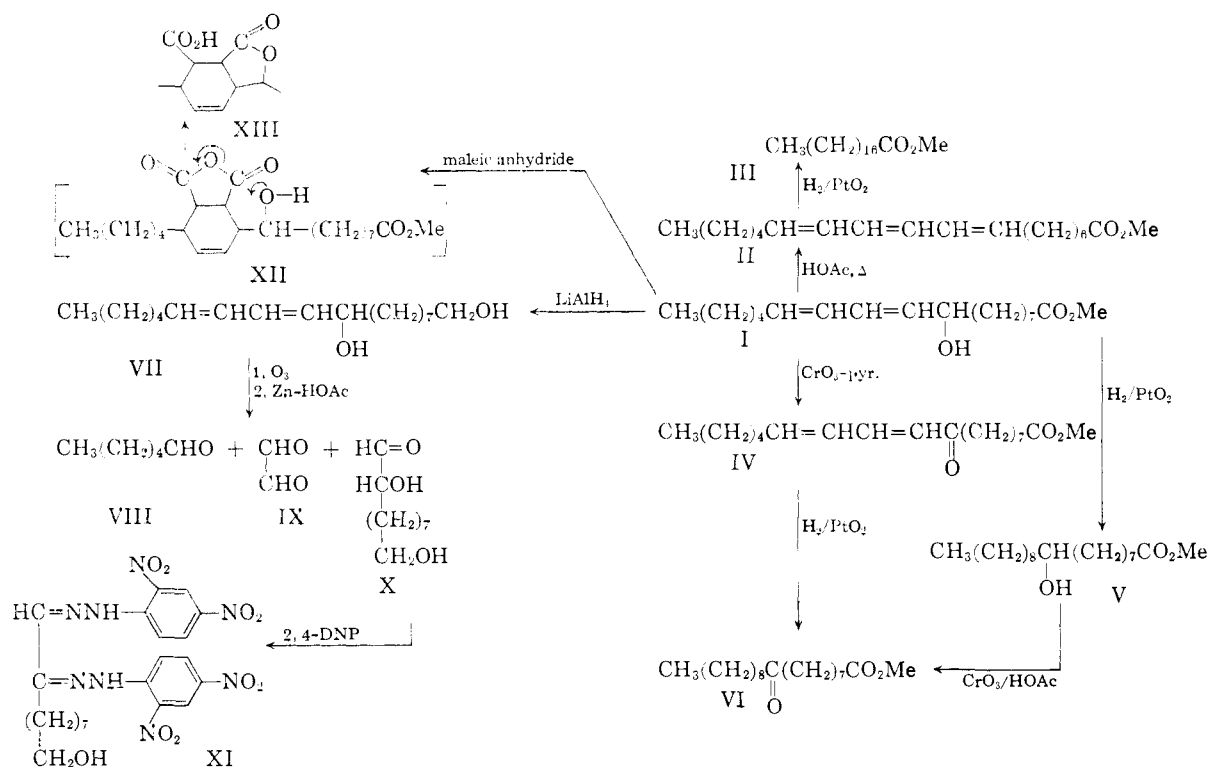
(4) C. R. Scholfield, J. Nowakowska and H. J. Dutton, Presentation before the 50th Spring Meeting, American Oil Chemists' Society, New Orleans, La., April 20–22, 1959.

(5) J. E. Jackson, R. F. Paschke, W. Tolberg, H. M. Boyd and D. H. Wheeler, *J. Am. Oil Chemists' Soc.*, **29**, 229 (1952); N. H. E. Ahlers, R. A. Brett and N. G. McTaggart, *J. Appl. Chem. (London)*, **3**, 433 (1953); D. H. Wheeler in "Progress in the Chemistry of Fats and Other Lipids," Vol. 2, ed. by R. T. Holman, *et al.*, Pergamon Press, London, 1954, p. 268.

(6) A. J. Durbetaki, *Anal. Chem.*, **28**, 2000 (1956).

(7) M. C. Burnett, unpublished data; for analytical methods used, cf. "Official and Tentative Methods of the American Oil Chemists' Society," Cd 7-48, revised April, 1956.

(8) F. D. Gunstone, *J. Chem. Soc.*, 1611 (1954).



an orange-red compound, m.p. 144–147°, tentatively formulated as a **dinitrophenylosazone** on the basis of elementary analyses and visible spectrum.

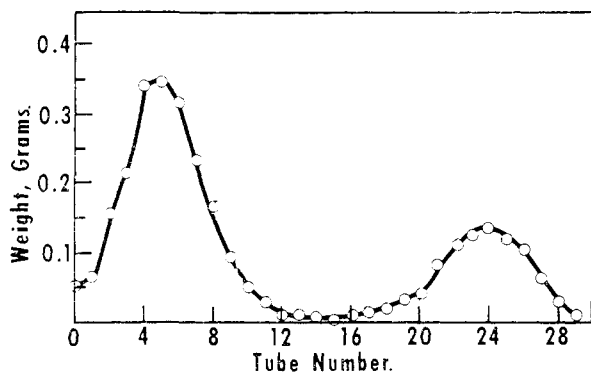


Fig. 1.—Weight distribution obtained in countercurrent distribution of mixed methyl esters, using hexane-acetonitrile.

Methyl dimorphecolate (I) on treatment with maleic anhydride readily formed a Diels-Alder adduct which was not obtained crystalline. Infrared spectra of the adduct showed maxima at 5.59 (γ -lactone), 5.73 (ester) and 5.82 μ (acid), but none ascribable to an anhydride. It is therefore suggested that the formally expected adduct may have rearranged to lactone XIII. Precedents for such a rearrangement are known. Heilbron and co-workers⁹ found that the maleic anhydride adducts of 3,5-hexadiene-2-ol and some related dienes were isolated as lactonic acids analogous to XIII.

On the basis of the evidence available, dimorphecolic acid can be assigned the structure I, that of 9-hydroxy-*trans,trans*-10,12-octadecadienoic acid.

(9) I. M. Heilbron, E. R. H. Jones, J. T. McCombie and B. C. I. Weedon, *J. Chem. Soc.*, 84 (1945).

The reasons for the high reactivity of I toward hydrogen bromide remain obscure. Spectral evidence suggests that hydrogen bromide reacts with I under the conditions of the oxirane oxygen determination⁶ by displacement of hydroxyl accompanied by some elimination. This allylic hydroxyl would be expected to be highly reactive, but further work will be required to elucidate fully the mechanism of this reaction. It is noteworthy in this connection that Bergström and Hansson¹⁰ treated methyl linoleate with N-bromosuccinimide and obtained initially a *conjugated dienoid* bromide. This product tended to eliminate hydrogen bromide to form a conjugated triene, but the elimination reaction *did not* go to completion.

Dimorphecolic acid appears to be unique in being the first naturally occurring fatty acid having more than 12 carbon atoms which is a conjugated diene.¹¹ It increases by one the list of previously known long-chain hydroxy acids of lipid origin.¹² It is apparent from the formulas of known C-18 fatty acids that dimorphecolic acid falls into a widespread biogenetic pattern, since the carbon atoms involved in centers of unsaturation or oxygenation are most frequently carbons 9 to 13. It differs

(10) S. Bergström and G. Hansson, *Acta Chem. Scand.*, **4**, 435 (1950).

(11) With the possible exception of 9,14-dihydroxy-10,12-octadecadienoic acid, which Davis, Conroy and Shakespeare (*cf. THIS JOURNAL*, **72**, 124 (1950)) isolated from tung oil, but considered to be an artifact. The longest chain conjugated dienoid fatty acid previously known to occur naturally is 2,4-dodecadienoic acid, isolated from *Sebastiania linguistrina* seed oil by Holman and Hanks (*cf. J. Am. Oil Chemists' Soc.*, **32**, 356 (1955)).

(12) For reviews of this subject, see (a) T. P. Hilditch, "Chemical Constitution of Natural Fats," 3rd Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 517; (b) F. D. Gunstone in "Progress in Organic Chemistry," Vol. IV, ed. by J. W. Cook, Academic Press, Inc., New York, N. Y., 1958, p. 14.

atmosphere with platinum oxide catalyst. The catalyst was removed by filtration and the solvent evaporated, yielding 0.319 g. of white solid (V), m.p. 43–48°. Two recrystallizations from hexane yielded 0.071 g. of V, m.p. 49–51°, whose infrared spectrum was very similar to that of (\pm)-methyl-9-hydroxyoctadecanoate, but markedly different from those of isomeric hydroxyoctadecanoates. This preparation was not readily reproducible, owing to the fact that compound I tended to suffer loss of hydroxyl. Yields were variable and generally very low. The preferred route for conversion of I to VI is through intermediate IV.

Anal. Calcd. for $C_{19}H_{38}O_3$: C, 72.6; H, 12.2; OMe, 9.8; C-Me, 1.0. Found: C, 72.3; H, 12.0; OMe, 9.8; C-Me, 0.9.

Oxidation of Methyl Dimorphecolate (I).—Compound (1.89 g.) in 10 ml. of dry pyridine was added to a solution of 2.0 g. of chromium trioxide in 20 ml. of dry pyridine.²⁰ The mixture was allowed to react 24 hours at room temperature with continuous stirring. The mixture then was diluted with water and extracted four times with ether. The combined ether extracts were washed with 5% hydrochloric acid, then with water, dried over sodium sulfate and evaporated. The yield was 1.42 g. of dark reddish oil, which was purified by chromatography on neutral alumina; 1.09 g. of purified IV, m.p. 26–28°, was obtained by benzene elution. A 0.169-g. portion of the eluate recrystallized from hexane yielded 0.039 g. having m.p. 28–30°. The ultraviolet spectrum showed λ_{\max} 275 m μ (ϵ 16,700); infrared maxima included 5.72 (ester), 5.90 (conjugated dienone²¹), 5.98 and 9.95 μ (conjugated dienone).

Anal. Calcd. for $C_{19}H_{38}O_3$: C, 74.0; H, 10.5. Found: C, 73.8; H, 10.4.

Preparation of Methyl 9-Oxoöctadecanoate (VI). A. By Oxidation of V.—A 0.220-g. portion of V was dissolved in 10 ml. of glacial acetic acid. To this was added, dropwise and with continuous stirring, 0.70 g. of chromium trioxide dissolved in 0.5 ml. of water and 8 ml. of acetic acid. The mixture was heated at 50° for 15 minutes, then cooled, diluted with water and extracted four times with ether. The combined ether extracts were washed with dilute sodium hydroxide, then with water and dried over sodium sulfate. Upon evaporation, 0.128 g. of VI was obtained, m.p. 44–45°. This was recrystallized from methanol, but without increase in melting point.

B. By Hydrogenation of IV.—A 0.276-g. portion of IV was hydrogenated in 30 ml. of absolute ethanol with 0.013 g. of platinum oxide catalyst for 30 minutes at 1 atmosphere and room temperature. A crude product, 0.267 g. of VI having m.p. 40–42°, was obtained. After two recrystallizations from hexane, 0.090 g. was obtained, m.p. 44–45° (lit.²² m.p. 47.5–48°). There was no depression of melting point on admixture of specimens prepared by methods A and B. The infrared spectrum of VI was identical with that of synthetic methyl 9-oxoöctadecanoate, but different from those of isomeric oxoöctadecanoates.

Anal. Calcd. for $C_{19}H_{36}O_3$: C, 73.0; H, 11.6. Found: C, 72.7; H, 11.5.

Lithium Aluminum Hydride Reduction of I.—Lithium aluminum hydride (2.22 g.), suspended in dry ether, was reacted dropwise with 2.08-g. of I, dissolved in dry ether. Spontaneous refluxing was at first maintained by controlling the rate of addition of I. Sufficient heat was applied to maintain reflux for 2.5 hours. Sufficient ethyl acetate was then added to decompose the excess lithium aluminum hydride. The mixture was extracted with 10% sodium hydroxide to remove inorganic matter. The remaining ethereal solution, after being dried with sodium sulfate and evaporated, yielded 1.57 g. of crude VII, m.p. 44–46°. This was recrystallized from hexane; 1.34 g., m.p. 46–47°, was obtained.²³ The ultraviolet spectrum of VII showed λ_{\max} 231 m μ (ϵ 19,360); infrared maxima included: 2.95 (hydroxyl, very strong), 10.12 μ (conjugated diene^{5,17,18}).

(20) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *This Journal*, **75**, 422 (1953).

(21) R. N. Jones, D. A. Ramsay, D. S. Kier and K. Dobriner, *ibid.*, **74**, 80 (1952).

(22) S. Bergström, G. Aulin-Erdtman, B. Rolander, E. Stenhagen and S. Östling, *Acta Chem. Scand.*, **6**, 1157 (1952).

(23) This 10,12-octadecadiene-1,9-diol should be analogous to, and isomeric with the diene-diol, m.p. 62.3–63°, obtained by lithium aluminum hydride reduction of ximenynolic acid (*cf.* ref. 13).

Anal. Calcd. for $C_{18}H_{34}O_2$: C, 76.5; H, 12.1. Found: C, 76.7; H, 11.9.

Ozonolysis of VII.—A 1.01-g. portion of VII (7.12 mmoles) was dissolved in 20 ml. of absolute methanol and ozonized at –35° with *ca.* 8.5 mmoles of ozone. The measured uptake of ozone was 7.28 mmoles. Ozonides were decomposed reductively by heating the mixture with 2 ml. of glacial acetic acid, followed by adding 1 g. of zinc dust in portions until a negative peroxide test was obtained. Zinc was then removed by filtration; the filtrate was diluted somewhat with water and extracted several times with petroleum ether.

The combined petroleum ether extracts were distilled cautiously to remove most of the solvent. The residue was steam distilled. A volatile aldehyde (VIII) was isolated from the steam distillate by ether extraction. The 2,4-dinitrophenylhydrazone of VIII was prepared by the method of Shriner, Fuson and Curtin.²⁴ This derivative had m.p. 94–97°; no depression of melting point was apparent on admixture with an authentic specimen of the 2,4-dinitrophenylhydrazone of hexanal. The identity of this derivative was confirmed by its X-ray diffraction pattern and by paper chromatography, using the solvent system heptane-methanol²⁵. The observed R_f value was 0.78 for both the 2,4-dinitrophenylhydrazone of VIII and authentic hexanal 2,4-dinitrophenylhydrazone; for heptanal 2,4-dinitrophenylhydrazone run simultaneously, the observed R_f was 0.82.

Anal. Calcd. for $C_{12}H_{16}N_4O_4$: C, 51.4; H, 5.8; N, 20.0. Found: C, 50.8; H, 5.8; N, 19.8.

The aqueous alcoholic residue left after petroleum ether extraction was saturated with sodium chloride, then extracted several times with ether. The combined, dried ether extracts were concentrated to 19 ml. and heated with a solution of 1.2 g. of 2,4-dinitrophenylhydrazine, 6 ml. of concd. sulfuric acid, 9 ml. of water and 20 ml. of ethanol.²⁴ An intractable mixture of 2,4-dinitrophenylhydrazones was obtained. By a combination of fractional crystallization and column chromatography (neutral alumina), two main fractions were obtained. One, a poorly crystalline orange substance, m.p. 144–147°, sparingly soluble in ethanol, was formulated tentatively as the dinitrophenylphenylosazone of 2,10-dihydroxydecanal (X); λ_{\max} 399 (ϵ 39,900) and 433 m μ (ϵ 40,800).²⁶

The other solid derivative, a brown substance, m.p. 280–320°, insoluble in chloroform and ethanol and moderately soluble in dioxane, was presumed to be the bis-dinitrophenylhydrazone of glyoxal.²⁷ It was not obtained in pure or well-crystallized form.

Maleic Anhydride Adduct of I.—A 0.314-g. portion of I (1.02 mmoles) and 0.110 g. of maleic anhydride were dissolved in 3 ml. of benzene and refluxed 3 hours. The benzene was evaporated and the residue dissolved in ether. The ether solution was extracted twice with water, then dried over sodium sulfate and evaporated, yielding 0.381 g. of clear oil. This adduct was not obtained crystalline. Its infrared spectrum was in agreement with a carboxylactone formulation (XIII) rather than an anhydride (XII); maxima were at 5.59 (γ -lactone), 5.73 (ester) and 5.82 μ (acid); it was transparent in the 231 m μ region of the ultraviolet spectrum.

Reaction of I and VII with Hydrogen Bromide.—Under conditions of Durbetaki's method for determining oxirane oxygen,⁸ compound I consumed 1.0 mole of hydrogen bromide; I (0.184 g., 5.72 mmoles) was dissolved in 10 ml. of benzene, then 10 ml. of hydrogen bromide-acetic acid reagent (5.72 mmoles) was added dropwise. The solvents were removed *in vacuo*. The infrared spectrum of the product showed practically no hydroxyl peak (2.94 μ); ultraviolet absorption spectra indicated disappearance of about half the absorbance of I at 231 m μ (conjugated diene) with appearance of a corresponding amount of triene (max. at 260, 268 and 279 m μ). Compound VII consumed 1.1 moles of

(24) R. L. Shriner, R. C. Fuson and D. Y. Curtin, "Systematic Identification of Organic Compounds," 4th ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 219.

(25) D. P. Meigh, *Nature*, **170**, 579 (1952).

(26) Glucose 2,4-dinitrophenylosazone has $\lambda_{\max}^{\text{dioxane}}$ 445 m μ (*cf.* A. L. Lehninger, *J. Biol. Chem.*, **149**, 43 (1943)).

(27) Literature m.p. 328° (*cf.* Shriner, Fuson and Curtin, *ref.* 24, p. 283).

hydrogen bromide per mole under conditions of the analytical method.⁶

Methylation of Acidic Material in Tubes 0-2, Countercurrent Distribution.—The material obtained in tubes 0-2 of the countercurrent distribution was a semi-solid. Its infrared spectrum was similar to that of methyl dimorphocolate (I), except that it had the characteristics of an acid rather than an ester. A 3.4-g. portion of this acidic material was dissolved in dry ether and added dropwise to a solution of 0.63 g. of diazomethane in 75 ml. of ether at room temperature. The excess diazomethane was removed by warming gently; on evaporation of solvent, an oily substance was obtained whose infrared spectrum was that of I.

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den, for correlating the infrared spectra of compounds V and VI with their synthetic materials. They also wish to thank Miss Janina Nowakowska of this Laboratory for gas chromatographic analysis of III; Mr. Henry Zobel for X-ray diffraction analysis of the 2,4-dinitrophenylhydrazone of VIII; Mr. C. R. Scholfield for valuable discussions of countercurrent distribution methods; Mr. Curtis Glass for infrared spectra; Mr. C. H. Van Etten for microanalyses; and Dr. Quentin Jones, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, for providing generous quantities of *Dimorphothea* seed.

PEORIA, ILL.

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND BACTERIOLOGY, STATE UNIVERSITY OF IOWA]

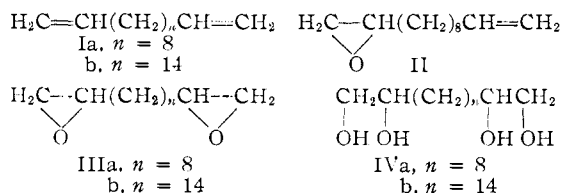
The Preparation and Properties of Some Oxidation Products of 1,11-Dodecadiene and 1,17-Octadecadiene¹

BY S. WAWZONEK, P. D. KLIMSTRA,^{2,3} R. E. KALLIO AND J. E. STEWART³

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1,11-Dodecadiene, 1,2-epoxy-11-dodecene, 1,2,11,12-diepoxydodecane, 1,2,11,12-tetrahydroxydodecane, 1,17-octadecadiene, 1,2,17,18-diepoxyoctadecane and 1,2,17,18-tetrahydroxyoctadecane have been prepared for testing as possible intermediates in the oxidation of dodecane and octadecane by microorganisms. The epoxidation of both olefins with perbenzoic acid proceeded stepwise. The reaction of 1,2,11,12-diepoxydodecane with sodium *p*-bromophenoxide gave 1,2-epoxy-11-hydroxy-12-*p*-bromophenoxydodecane and bis-1,12-*p*-bromophenoxy-2,11-dihydroxydodecane. Tests with yeast *Candida lipolytica* indicate that the various derivatives of dodecane are oxidized more slowly than oxidation products involving one end of the hydrocarbon.

In the study of the oxidation of saturated hydrocarbons by certain microorganisms, oxidation products involving both ends of the hydrocarbons were required for the elucidation of the path of the oxidation. Work in this study was limited to the oxidation products of dodecane and octadecane and included the preparation of 1,11-dodecadiene (Ia), 1,17-octadecadiene (Ib), 1,2-epoxy-11-dodecene (II), 1,2,11,12-diepoxydodecane (IIIa), 1,2,17,18-diepoxyoctadecane (IIIb), 1,2,11,12-tetrahydroxydodecane (IVa) and 1,2,17,18-tetrahydroxyoctadecane (IVb).



Both dienes were obtained from the action of allyl chloride on the Grignard reagent from 1,6-dichlorohexane.⁴ Purification of the dienes was accomplished through the formation of the silver nitrate complexes and was necessary for 1,11-dodecadiene since this compound co-distilled with 1,6-dichlorohexane.

(1) This research was supported by a grant from the Petroleum Research Fund administered by the American Chemical Society. Grateful acknowledgment is hereby made to the donor of said fund.

(2) Abstracted in part from the M.S. thesis, February, 1957, and the Ph.D. thesis, June, 1959, of P. D. Klimstra.

(3) American Chemical Society Petroleum Research Fund Pre-doctoral Fellows.

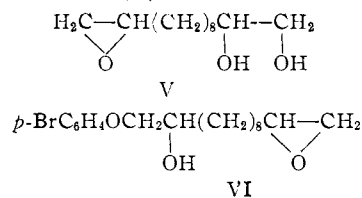
(4) F. Drahowzal, *Monatsh.*, **82**, 793 (1951).

The preparation of 1,2,11,12-diepoxydodecane (IIIa) from the bis-chlorohydrin was not successful. The bis compound, which was not obtained pure, was resistant to cyclization with cold alkali.

Direct epoxidation of 1,11-dodecadiene (Ia) with peracetic acid gave 1,2,11,12-diepoxydodecane (IIIa). Better yields and less side products were obtained, however, using perbenzoic acid. The epoxidation at low temperatures was found to proceed stepwise with both reagents. Using an excess of perbenzoic acid one mole of the reagent added at 5-6° in less than two hours while the second mole required about ten hours to react (see Fig. 1).

A similar stepwise addition of perbenzoic acid occurred with 1,17-octadecadiene (Ib) and 1,5-hexadiene and is shown in Fig. 1.

By interrupting the reaction with peracetic acid and 1,11-dodecadiene before it had gone to completion it was possible to isolate both the monoepoxide II and diepoxy IIIa together with a fraction which had the composition expected for 1,2-epoxy-11,12-dihydroxydodecane (V).



To determine whether this stepwise addition was characteristic of other reagents, the addition of bromine was studied with both 1,11-dodecadiene (Ia) and 1,17-octadecadiene (Ib) and found to be