

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

Ring Enlargements. II. Attempted Ring Enlargement of Cyclohexanone with Ethyl N-Nitroso-N-(2,3,4-trimethoxybenzyl)-carbamate

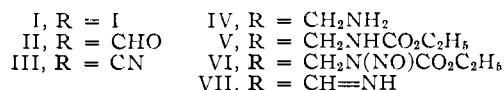
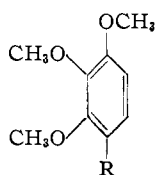
BY C. DAVID GUTSCHE AND HERBERT E. JOHNSON¹

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An attempt has been made to prepare 2-(2',3',4'-trimethoxyphenyl)-cycloheptanone by ring enlargement of cyclohexanone with ethyl N-nitroso-N-(2,3,4-trimethoxybenzyl)-carbamate. The reaction, however, took a different course and yielded 2,3,4-trimethoxybenzyl methyl ether in the presence of methanol, 2,3,4-trimethoxybenzyl ethyl carbonate in the absence of an alcohol and a mixture of the carbonate and 2,3,4-trimethoxybenzyl *t*-butyl ether in the presence of *t*-butyl alcohol. A two-step intramolecular rearrangement has been postulated to explain the formation of the carbonate.

In the first paper of this series² it was shown that 2-phenylcycloheptanone could be obtained from cyclohexanone and ethyl N-nitroso-N-benzylcarbamate in the presence of methanol and potassium carbonate. With the intention of studying the scope of this type of ring enlargement reaction and in the hope of developing a good method for the synthesis of 2-(2',3',4'-trimethoxyphenyl)-cycloheptanone, a starting material of interest in colchicine syntheses,³ experiments were carried out with ethyl N-nitroso-N-(2,3,4-trimethoxybenzyl)-carbamate (VI).

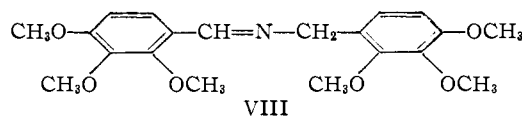
Preparation of Ethyl N-Nitroso-N-(2,3,4-trimethoxybenzyl)-carbamate (VI).—Two routes to the necessary intermediate, 2,3,4-trimethoxybenzylamine (IV), were investigated, both starting with 1,2,3-trimethoxybenzene. According to the first route trimethoxybenzene was converted to 2,3,4-trimethoxybenzaldehyde (II) by the procedure of Wallenfells,⁴ the aldehyde was converted to the aldoxime, and the aldoxime was catalytically reduced to the amine IV. The over-all yield, however, proved to be unsatisfactory due to generally poor and capricious results at the formylation step and to the formation of appreciable amounts of secondary amine in the reduction step.



The second, and more satisfactory, route consisted in the conversion of trimethoxybenzene to 2,3,4-trimethoxyiodobenzene (I) by the method of Baker, *et al.*,⁵ replacement of the iodine by a cyano group by the action of cuprous cyanide, and reduction of the nitrile III to the amine IV with lithium aluminum hydride. Under the usual conditions the cyanation reaction was difficult to control and often commenced with explosive violence. The addition of previously prepared nitrile to the initial reaction mixture, however, prevented this and al-

lowed a smooth conversion of I to III in 90% yield.⁶

Although compounds such as benzonitrile and *o*-toluonitrile have been reduced smoothly and in good yield to the corresponding primary amines by means of lithium aluminum hydride,⁷ 2,3,4-trimethoxybenzonitrile (III) failed to follow this pattern. Even under the optimum conditions, based in part on recently published considerations of concentration and temperature effects,⁸ only 65–68% of basic material could be obtained, and it consisted of a mixture of the amine IV and the aldimine VII. Removal of the aldimine by subjecting the crude basic fraction to a short acid hydrolysis (VII converted to II) left IV as the only basic component in, at best, 50% yield. From reaction mixtures that had stood at room temperature for several days there was isolated the Schiff base VIII, the structure of which was proved by a synthesis from II and IV. The greater tendency to aldimine formation in III as compared with benzonitrile may probably be ascribed to greater insolubility of the intermediate products from III and to the strong neutralizing effect of the *o*- and *p*-methoxyl groups on the electron deficient carbon atom of the nitrile group (presumably the point of nucleophilic attack by an aluminohydride anion).



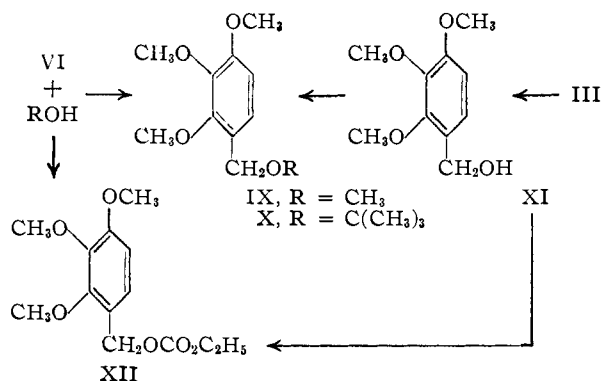
Conversion of IV to the urethan V and the nitroso compound VI offered little difficulty and proceeded in almost quantitative yield.

Reactions of Ethyl N-Nitroso-N-(2,3,4-trimethoxybenzyl)-carbamate (VI).—When VI was added to a mixture of methanol, cyclohexanone and finely powdered potassium carbonate, nitrogen was evolved smoothly, rapidly and in quantitative amount, but the product showed no trace of the desired ketone. Instead, there was obtained a 72% yield of 2,3,4-trimethoxybenzyl methyl ether (IX), the structure of which was proved by carbon, hydrogen and methoxyl analysis, by bromination to a tribromo derivative (probably 1,2-dibromo-3,4,5-trimethoxybenzyl bromide) and by an unequivocal

(1) Petrolite Corporation Fellow, 1952–1953.
 (2) C. D. Gutsche, *This Journal*, **71**, 3513 (1949).
 (3) C. D. Gutsche and F. A. Fleming, *ibid.*, **76**, 1771 (1954).
 (4) K. Wallenfells, *Ber.*, **74B**, 1428 (1941).
 (5) W. Baker, A. W. W. Kirby and L. V. Montgomery, *J. Chem. Soc.*, 2876 (1932).

(6) C. F. Koelsch and A. G. Whitney, *J. Org. Chem.*, **6**, 795 (1941), showed that the cyanation reaction is autocatalytic.
 (7) R. F. Nyström and W. G. Brown, *This Journal*, **70**, 3737 (1948).
 (8) L. H. Admundsen and L. S. Nelson, *ibid.*, **73**, 242 (1951); H. R. Nace and B. B. Smith, *ibid.*, **74**, 1861 (1952); H. E. Zaugg and B. W. Harrom, *ibid.*, **75**, 292 (1953).

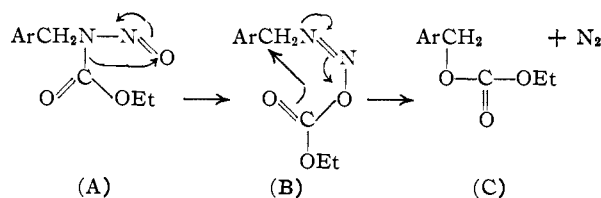
synthesis from the sodio derivative of 2,3,4-trimethoxybenzyl alcohol (XI) and methyl iodide.



With cyclohexanone as the solvent and with only a trace of methanol present, VI failed to react at room temperature but at an elevated temperature evolved nitrogen in quantitative amount. The product from this reaction was neither the desired ketone nor the ether IX but the unsymmetrical carbonate XII, the structure of which was proved by carbon, hydrogen and methoxyl-ethoxyl analyses, by the infrared spectrum, and by an unequivocal synthesis from XI and ethyl chloroformate. Neither cyclohexanone, methanol, nor a base was necessary for the formation of XII, however, for it resulted in equally high yield (70–75%) when VI was heated in cyclohexanone or nitrobenzene solution alone.

From a reaction carried out in refluxing *t*-butyl alcohol in the presence of potassium carbonate there was isolated 44% of the carbonate XII and 30% of 2,3,4-trimethoxybenzyl *t*-butyl ether (X).

Discussion of Results.—The preference demonstrated by VI to react with alcohols instead of ketones will be discussed in a future paper, and only the formation of the carbonate will be considered here. Bollinger, Hayes and Siegel⁹ have studied the decomposition of *N*-nitroso-*N*-cyclohexylurethan and *N*-nitroso-*N*-cyclopentylurethan in methanol in the presence of potassium carbonate and have isolated *inter alia* ethyl cyclohexyl carbonate and ethyl cyclopentyl carbonate. The mechanism invoked for the rationalization of the products of decomposition assumed cyclohexane- and cyclopentane diazonium ions as intermediates. Such an assumption appears unnecessary in the present case, however, and we propose that XII arises from VI by two consecutive intramolecular rearrangements as indicated in the sequence



The conversion from A to B has been well-substantiated in the related compound *N*-nitrosoacetanil-

(9) F. W. Bollinger, F. N. Hayes and S. Siegel, *THIS JOURNAL*, **72**, 5592 (1950); *ibid.*, **75**, 1729 (1953).

ide¹⁰; the conversion from B to C is merely an hypothesis based on the possibility of a two-step conversion as indicated by the kinetic data for the reaction. The reaction of VI with an alcohol to form the benzyl ether may or may not involve B as an intermediate but in any event appears to go through the diazoalkane as suggested by the colors observed during the various experiments. Those reaction mixtures in which the carbonate was the sole product remained pale yellow throughout; the reaction mixture of VI in methanol which gave the ether as the sole product became very deep red at the beginning and then faded to yellow; the reaction mixture of VI in *t*-butyl alcohol which gave a mixture of the carbonate and the ether developed a color intermediate between these two extremes.

Experimental^{11,12}

2,3,4-Trimethoxybenzonitrile (III).—The procedure employed by Callen, *et al.*,¹³ for the preparation of 9-cyanophenanthrene was followed with certain modifications. A mixture of 442 g. (1.5 moles) of 2,3,4-trimethoxyiodobenzene (m.p. 40–42°) and 172 g. (1.9 moles) of cuprous cyanide was prepared. A 20-g. portion of this mixture and 5 g. of previously prepared product¹⁴ was placed in a 500-ml. round-bottomed flask equipped with a thermometer and an efficient stirrer. The flask was heated slowly, with stirring, until the temperature was *ca.* 200°. At this point the melt became dark colored and mobile, and the temperature rapidly rose to 290°. When the temperature had fallen to 240° the remainder of the mixture was added portionwise, over a period of 1 hour, maintaining the temperature at *ca.* 250°. The reaction mixture was stirred and heated an additional 30 minutes, the flask was cooled, and the product was extracted into a total of 1000 ml. of boiling benzene, added portionwise. The benzene was removed by evaporation, and the residue was distilled to yield 257 g. (89% corrected for III added) of a glistening white solid, m.p. 52–55°, b.p. 164–167° (8 mm.). Higher yields can be obtained (94%) by distilling the product directly from the original reaction flask, but the product so obtained is somewhat less pure (m.p. 47–52°).

2,3,4-Trimethoxybenzylamine (IV). (A) From 2,3,4-Trimethoxybenzonitrile (III).—To a rapidly stirred solution of 21.2 g. (0.562 mole) of lithium aluminum hydride in 1200 ml. of absolute ether cooled to 3–5° was added, over a period of 1.75 hours, 51.0 g. (0.264 mole) of 2,3,4-trimethoxybenzonitrile (m.p. 52–55°) in 500 ml. of absolute ether. The reaction mixture containing a suspension of white, granular material was stirred at 3° for an additional 30 minutes, and it was then decomposed with methanol followed by water and a 20% solution of potassium sodium tartrate. The supernatant liquid was decanted from the precipitate, the precipitate was washed with 100 ml. of ether, and the combined ether solution was extracted several times with 1:10 hydrochloric acid. From the acid extract there was obtained 34.5 g. (66.5%) of an almost colorless oil, *n*_D²⁵ 1.5545. This material was heated for 10 minutes with dilute (1:5) hydrochloric acid, the aldehyde formed was extracted into warm benzene, and the amine was isolated in the usual way and distilled to give 26.0 g. (50%) of a colorless oil, b.p. 87–89° (0.2 mm.), *n*_D²⁵ 1.5340. A pi-

(10) For articles concerning this *cf.* D. H. Hey, J. Stuart-Webb and G. H. Williams, *J. Chem. Soc.*, 4657 (1952); D. F. DeTar, *THIS JOURNAL*, **73**, 1446 (1951); von R. Huisgen and G. Horeld, *Ann.*, **562**, 137 (1949).

(11) All melting points are corrected; all boiling points are uncorrected.

(12) We are indebted to Mr. William Parr for most of the microanalyses.

(13) J. E. Callen, C. A. Dornfeld and G. H. Coleman, *Org. Syntheses*, **28**, 34 (1948).

(14) The reaction can, of course, be carried out without the use of previously prepared product. The initiation of the reaction, however, is much smoother in its presence, and it is therefore advisable to carry out a small run in order to procure a sample of III for use in larger runs.

crate of m.p. 211–213° dec. was obtained easily (reported¹⁵ 208–210° dec.).

From some reaction mixtures (run under sub-optimal conditions) that were allowed to stand at room temperature for a day or more there was obtained a solid, m.p. 42–62°. Several recrystallizations from aqueous methanol produced colorless needles, m.p. 71–72°, soluble in 5% hydrochloric acid, showing a neutral equivalent (to acid) of ca. 400, and giving negative reactions in primary amine tests.

Anal. Calcd. for $C_{20}H_{25}NO_6$: C, 63.99; H, 6.71. Found: C, 63.93; H, 6.65.

Hydrolysis of the product was effected by heating at 60° with 10% hydrochloric acid. The neutral fraction yielded an oxime, m.p. 92–93°, identical with that of II, and the basic fraction yielded a picrate, m.p. 210–212°, identical with that from IV. The same compound (VIII) also was obtained by allowing a mixture of 1.15 g. of IV and 1.15 g. of II to stand for one week over phosphorus pentoxide in an evacuated desiccator. The crude product (2.30 g.), m.p. 68–71°, yielded colorless needles upon recrystallization from aqueous methanol, m.p. 71–72°.

(B) From 2,3,4-Trimethoxybenzaldehyde (II).—2,3,4-Trimethoxybenzaldehyde was converted in 99% yield to the oxime, m.p. 78–88°. One recrystallization yielded glistening plates, m.p. 92–93° (reported¹⁶ 91°) of sufficient purity for catalytic reduction. A 20.0-g. sample of the pure oxime was dissolved in absolute methanol and hydrogenated at 3 atmospheres in the presence of 4 g. of 10% palladium chloride-on-charcoal catalyst. The acid-soluble fraction consisted of 16.0 g. (86%) of a greenish oil which was not pure but consisted *inter alia* of the desired amine IV and the secondary amine. Vacuum distillation achieved a separation, but yielded only 26% of IV.

Ethyl N-(2,3,4-trimethoxybenzyl)-carbamate (V) was prepared by the general method described by Hartman and Brethen.¹⁷ Removal of the ether from the product yielded 97% of a colorless solid; m.p. 52–55°. Several recrystallizations from petroleum ether (b.p. 63–69°)—ethyl acetate gave colorless needles, m.p. 56.5–57.5°.

Anal. Calcd. for $C_{13}H_{19}NO_5$: C, 57.98; H, 7.11. Found: C, 57.71; H, 7.09.

Ethyl N-nitroso-N-(2,3,4-trimethoxybenzyl)-carbamate (VI) was prepared from V (m.p. 51–54°) by the method of Hartman and Phillips.¹⁸ The nitrosation was carried out at 15–20° for a period of 6 hours and yielded an orange-red oil in quantitative amount which was not purified but was used directly in further reactions. On the basis of the nitrogen evolved in subsequent reactions, this product contained ca. 100% of the N-nitroso compound. The reported instability of trimethoxyphenyl systems in the presence of nitrous acid¹⁹ was not apparent under these conditions.

Reaction of VI in Methanol Solution.—A stirred mixture of 10.0 g. (0.102 mole) of cyclohexanone, 50 ml. of absolute methanol, and 1 g. of finely powdered potassium carbonate was treated, over a period of 10 minutes, with 10.0 g. (0.0335 mole) of VI, the reaction temperature being maintained at 25–30°. The product was isolated in the usual fashion and was fractionally distilled to yield 5.02 g. (72%) of a colorless liquid, b.p. 83–84° (0.3 mm.), n_D^{25} 1.5135; $\lambda_{max}^{CHCl_3}$ in cm^{-1} 943, 986, 1010, 1042, 1098, 1127, 1150, 1197, 1273, 1375, 1410, 1465, 1492, 1600, 2825, 2925.²⁰ The physical and chemical data for this material are in accord with 2,3,4-trimethoxybenzyl methyl ether (IX).

Anal. Calcd. for $C_{11}H_{16}O_4$: C, 62.26; H, 7.61; CH_3O , 58.5. Found: C, 62.17; H, 7.39; CH_3O , 58.5.

A tribromo derivative from 2,3,4-trimethoxybenzyl methyl ether was prepared according to a procedure described by Shriner and Fuson²¹ and was obtained after several recrystallizations from petroleum ether (b.p. 63–69°) at –20° as colorless rhombs, m.p. 83–84°.

(15) L. Monti and G. Verona, *Gazz. chim. ital.*, **60**, 777 (1930).

(16) A. Heffter and R. Capellmann, *Ber.*, **38**, 3638 (1905).

(17) W. W. Hartman and M. R. Brethen, *Org. Syntheses*, **12**, 38 (1932).

(18) W. W. Hartman and R. Phillips, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 464.

(19) G. Graebe and H. Hess, *Ann.*, **340**, 232 (1905).

(20) We are indebted to Dr. Verner L. Stromberg of the National Heart Institute, Bethesda, Md., for the infrared spectra.

(21) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 189.

Anal. Calcd. for $C_{10}H_{11}Br_3O_3$: C, 28.66; H, 2.65; CH_3O , 22.2. Found: C, 28.76; H, 2.51; CH_3O , 22.5.

2,3,4-Trimethoxybenzyl methyl ether (IX) was prepared, for comparison with the material described above, as follows. Alkaline hydrolysis of 2,3,4-trimethoxybenzotrile (III) yielded 91% of 2,3,4-trimethoxybenzoic acid which was reduced with lithium aluminum hydride in 73% yield to 2,3,4-trimethoxybenzyl alcohol (XI), b.p. 105° (0.25 mm.). n_D^{25} 1.5348.

Anal. Calcd. for $C_{10}H_{14}O_4$: C, 60.60; H, 7.12. Found: C, 60.66; H, 7.04.

The 3,5-dinitrobenzoate of XI was obtained as feathery, yellow-green needles after several recrystallizations from methanol, m.p. 107.5–108.5°.

Anal. Calcd. for $C_{17}H_{16}N_2O_9$: C, 52.04; H, 4.12. Found: C, 52.00; H, 4.16.

Bromination of XI yielded the same tribromo derivative, m.p. 82–83.5°, that was obtained from IX. The sodio derivative of XI was prepared and was treated with methyl iodide in refluxing toluene to yield, after distillation, 76% of 2,3,4-trimethoxybenzyl methyl ether, b.p. 76–79° (0.25 mm.), n_D^{25} 1.5142, $\lambda_{max}^{CHCl_3}$ identical with that recorded above.

Reaction of VI in Cyclohexanone Solution.—A stirred mixture of 100 ml. of cyclohexanone, 1.5 ml. of absolute methanol and 1.0 g. of finely powdered potassium carbonate was heated to 114° and was treated, over a period of 5 hours, with 36.4 g. of VI. After 10 hours the evolution of nitrogen ceased and the reaction mixture was worked up in the usual fashion to yield, after distillation, 24.8 g. (75.5%) of a colorless oil, b.p. 128–131° (0.25 mm.), n_D^{25} 1.5023; $\lambda_{max}^{CHCl_3}$ in cm^{-1} 868, 903, 942, 1045, 1095, 1160, 1190–1275, 1360, 1377, 1410, 1465, 1492, 1600, 1735, 2825, 2925.²⁰

The physical and chemical data for this material are in accord with those of an authentic sample of 2,3,4-trimethoxybenzyl ethyl carbonate (XII) (*cf.* below).

Anal. Calcd. for $C_{13}H_{18}O_6$: C, 57.77; H, 6.71; Zeisel values, 46.2. Found: C, 57.44; H, 6.43; Zeisel value, 46.2.

The same material was obtained in essentially the same yield from the reaction of VI in cyclohexanone or nitrobenzene in the absence of any base or alcohol.

A piperidino derivative of XII was prepared by refluxing 2.00 g. of XII with 5 ml. of piperidine for 3 hours. The acid-soluble fraction of the product, consisting of 1.30 g. of an oil, was converted to the hydrochloride, recrystallized several times from ethyl acetate–petroleum ether (b.p. 88–98°), and obtained as glistening, colorless plates, m.p. 156–158°.

Anal. Calcd. for $C_{15}H_{23}ClNO_3$: C, 59.69; H, 8.02. Found: C, 59.38; H, 8.03.

Bromination of XII gave 100% yield of the same tribromo that was obtained from 2,3,4-trimethoxybenzyl methyl ether.

2,3,4-Trimethoxybenzyl Ethyl Carbonate (XII).—To a stirred solution of 7.60 g. (0.038 mole) of 2,3,4-trimethoxybenzyl alcohol in 100 ml. of absolute ether was added 10 g. of anhydrous potassium carbonate followed by 5.00 g. (0.045 mole) of ethyl chlorocarbonate. After 4 hours at room temperature the solids were removed by filtration, the ether was evaporated and the residue was distilled to yield 3.60 g. of starting material, 1.62 g. with b.p. 121–126° (0.40 mm.), and 4.04 g. with b.p. 200–210° (0.40 mm.). The material with b.p. 121–126° (0.40 mm.) was redistilled to give a colorless oil, b.p. 120° (0.25 mm.), n_D^{25} 1.5020, $\lambda_{max}^{CHCl_3}$ identical to that recorded above. The higher boiling fraction was redistilled to give a colorless oil, probably di-(2,3,4-trimethoxybenzyl) ether, b.p. 208–210° (0.30 mm.), n_D^{25} 1.5480; $\lambda_{max}^{CHCl_3}$ in cm^{-1} 802, 935, 1012, 1035, 1098, 1160, 1200–1240, 1268, 1352, 1379, 1415, 1460, 1495, 1600, 1732 (very weak), 2820, 2920, 3350.²⁰

Anal. Calcd. for $C_{20}H_{26}O_7$: C, 63.47; H, 6.93; CH_3O , 49.4. Found: C, 63.39; H, 6.89; CH_3O , 48.7.

Reaction of VI in *t*-Butyl Alcohol.—A mixture containing 50 ml. of anhydrous *t*-butyl alcohol, 3.42 g. (0.035 mole) of cyclohexanone, 10.0 g. (0.035 mole) of VI and 1.0 g. of finely powdered potassium carbonate was refluxed until the evolution of nitrogen ceased (20 hours). The reaction mixture was worked up in the usual way to yield 3.94 g. (44%) of 2,3,4-trimethoxybenzyl ethyl carbonate (XII) and 2.64

g. (30%) of 2,3,4-trimethoxybenzyl *t*-butyl ether (X) as a colorless oil, b.p. 91° (0.20 mm.), n_D^{25} 1.5043.

Anal. Calcd. for $C_{14}H_{22}O_4$: C, 66.11; H, 8.72. Found: C, 66.01; H, 8.38.

Kinetic Data.—A reaction of 10.0 g. (0.0335 mole) of VI in 30.0 ml. of cyclohexanone at $114 \pm 1^\circ$ showed the following nitrogen evolution: time in minutes (volume of nitrogen in ml.); 5(42), 10(110), 15(175), 20(230), 25(290), 30(345), 35(390), 40(430), 50(500), 60(545), 80(615), 120

(705), 240(775). A similar experiment with only 19 ml. of cyclohexanone gave essentially identical results. A plot of these data in terms of % reaction vs. log time gives a curve almost identical with that constructed from the equation for series first-order reactions in which it is assumed that k_1 and k_2 are approximately equal.²²

(22) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, pp. 153-157.
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[CONTRIBUTION FROM THE HORMEL INSTITUTE, UNIVERSITY OF MINNESOTA]

Displacement Analysis of Lipids. IX. Products of the Oxidation of Methyl Linoleate¹

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The peroxides formed by six different means of oxidation of methyl linoleate have been isolated, reduced to the corresponding hydroxy compounds and subjected to displacement analysis. The products obtained *via* autoxidation at -10° in the dark, with copper catalyst, with visible light irradiation, or with ultraviolet light irradiation are qualitatively similar. The two major dienoic reduction products exhibit *cis-trans* and *trans-trans* conjugation. With chlorophyll photooxidation, four major reduction products were found. One was found to exhibit no conjugation, and to have hydroxyl groups and isolated *trans* double bonds. It was found to have two double bonds and one hydroxyl group per molecule. This compound could be 11-hydroxylinoleate derived from the non-conjugated peroxide. The products of oxidation produced by lipoxidase oxidation to a level of 25% were found to consist of at least six substances distinguishable by displacement chromatography diagram. It is concluded that displacement analysis is a sharp analytical tool for the study of the products of fat oxidation.

Recent studies of the oxidation of esters of linoleic acid have shown that the primary product of oxidation probably exists as more than one isomer.² In the most generally accepted mechanism of oxidation of linoleate the free radical formed by removal of a labile hydrogen atom at carbon 11 exists as a resonance hybrid to which addition of oxygen takes place at the ends of the resonating system. This addition of oxygen leads to formation of hydroperoxides containing conjugated double bond systems. Current theories of oxidation of linoleate have been summarized recently.³ Although no direct evidence has been reported for the existence of non-conjugated peroxide formed by oxidative attack at carbon 11, its existence has not been ruled out. Recently the presence of such non-conjugated peroxides among the products of oxidation of linoleate was suggested by infrared spectra of peroxide concentrates obtained under certain conditions of oxidation.⁴

In the field of lipid analysis, displacement chromatography has been shown to be very useful for the small-scale separation of closely related substances. The applications of displacement analysis to lipids have been summarized recently.⁵ Briefly stated, long chain aliphatic compounds have been shown to be separable if they differ from each other by one carbon atom in length,⁶ type of functional group,^{7a} kind and degree of unsaturation^{7b} and by branching

or polar groups along the chain. It seemed likely then that displacement analysis might be used to separate the closely related products of oxidation of methyl linoleate, and that displacement diagrams of these products might yield additional information regarding the complexity of these products formed under differing conditions of oxidation. Moreover, isolation and characterization of the suspected non-conjugated peroxide might be possible.

Experimental

Methyl linoleate, prepared by bromination-debromination, was obtained from the Hormel Foundation. Methyl esters of corn oil acids were prepared by methanolysis and used for confirmatory experiments. Oxidation of these ester preparations was performed under six differing conditions: (A) autoxidation at -10° in darkness, (B) autoxidation with copper oleate catalyst, (C) autoxidation under visible light irradiation, (D) autoxidation under ultraviolet light irradiation, (E) oxidation in the presence of chlorophyll and under visible irradiation and (F) "enzymatic" oxidation with lipoxidase. The details of these oxidations have been described elsewhere.^{4,8} Analytical data pertinent to these preparations and later treatment are summarized in Table I. The linoleate which had been oxidized by various means was subjected to countercurrent extraction to isolate the peroxide components.⁹ These peroxide concentrates were then reduced by stannous chloride to obtain the monohydroxylinoleate preparations which were subjected to displacement chromatography.

The chromatographic apparatus and its use have been described in detail elsewhere.¹⁰ The system used in the separations reported here consisted of a coupled filter column of 76.2 ml. total volume packed with Darco-G60 and Hyflo Supercel, 1:2. The solvent was 95% ethanol and the displacer solution was 1.0% ethyl stearate. Samples of reduced oxidation products varied from 250 to 440 mg. The effluent from the chromatographic column in all cases, except C, was passed through a quartz flowing cell having a cell thickness of 0.2 mm. mounted in the cell holder of a Beckman DU spectrophotometer. Ultraviolet light absorption was measured at 2520 Å., which represents principally end

(1) Supported in part by contract with the Office of Naval Research (N8onr 66218), Department of the Navy. Hormel Institute publication no. 97.

(2) O. S. Privett, W. O. Lundberg, N. A. Khan, W. E. Tolberg and D. H. Wheeler, *J. Am. Oil Chem. Soc.*, **30**, 61 (1953).

(3) R. T. Holman, "Progress in the Chemistry of Fats and Other Lipids," Vol. II, Pergamon Press, London, 1954, p. 51.

(4) N. A. Khan, W. E. Tolberg, D. H. Wheeler and W. O. Lundberg, *J. Am. Oil Chem. Soc.*, in press.

(5) R. T. Holman, "Progress in the Chemistry of Fats and Other Lipids," Vol. I, Pergamon Press, London, 1952, p. 104.

(6) R. T. Holman and L. Hagdahl, *J. Biol. Chem.*, **182**, 421 (1950).

(7) (a) R. T. Holman, *This Journal*, **73**, 3337 (1951); (b) R. T. Holman and W. T. Williams, *ibid.*, **73**, 5285 (1951).

(8) N. A. Khan and W. O. Lundberg, *J. Am. Oil Chem. Soc.*, in press.

(9) O. S. Privett, W. O. Lundberg and C. Nickell, *ibid.*, **30**, 17 (1953).

(10) R. T. Holman and L. Hagdahl, *Anal. Chem.*, **23**, 794 (1951).