alcohol (n^{20}_{D} 1.3999, d^{20}_{4} 0.814) distilled over. Unchanged terpenes and butyl borate were removed at 100° and 1 mm. pressure, leaving 8.0 g. of non-volatile residue. This material was steam-distilled from 1 g. of sodium hydroxide and the resulting oil layer redistilled at 10 mm. to give 4.35 g. of optically inactive alcohol, b.p. 10 mm. 189–192°, n^{20}_{D} 1.4806, d^{20}_{4} 0.942.

The alcoholate, prepared by refluxing 0.5 g. of the compound in 4 ml. of benzene with excess sodium for half an hour, was decanted into a solution of 0.6 g. of *p*-nitrobenzoyl chloride in benzene. The ester obtained by evaporating the benzene melted at 55.5–57° after crystallization from methanol.

l-**Thujone.**—A solution of 10 g. each of fraction 38, semicarbazide hydrochloride, and sodium acetate in 70 ml. of 70% ethanol was allowed to stand for three days at room temperature. Solvent and 2.9 g. of unchanged terpenes were removed in a current of steam, leaving 12.0 g. of mixed semicarbazones. The alcohol insoluble portion was crystallized from water to give 0.5 g. of white microcrystalline powder m.p. 251–253° which was not decomposed by the action of oxalic acid of 2 *N* sulfuric acid at 100°.

From the fraction slightly soluble in ethanol was obtained 0.5 g. of a pure compound m.p. 193°, $[\alpha]^{27}_{D}$ +57.1 (c = 4.763 in methanol), which was probably *l*-thujone semicarbazone.

The fraction soluble in cold ethanol was crystallized from aqueous ethanol to give a partial separation of two compounds. One formed anisotropic needles m.p. 176° which may have been *d*-isothujone semicarbazone. The other was an isotropic powder m.p. about 128°.

When the latter three compounds were steam distilled from an equal weight of oxalic acid, ketones were obtained with refractive indices n^{20}_{D} 1.4496, 1.4502 and 1.4509, respectively. The latter two ketones were combined and oxidized according to Tiemann and Semmler.[12] From 0.5 g. of oil, 0.36 g. of α-thujaketonic acid, m.p. 74.5–75.5°, was obtained.

l-**Bornyl Acetate.**—Twenty grams of fraction 48 (n^{20}_{D} 1.4637, d^{20}_{4} 0.985, α_{D} −18.68) was boiled under reflux for 15 minutes with 60 ml. of 20% alcoholic potassium hydroxide, and poured into 250 ml. of water. The precipitate was crystallized from hexane to give crude borneol in a yield of 59%. The aqueous liquors were treated with excess carbon dioxide and evaporated to dryness. 9.2 g. of organic salts were leached from the residue with boiling alcohol. A mixture of 4 g. of the salt, 10 ml. of aniline, and 3 ml. of 35% hydrochloric acid was heated at 150–160° for one hour, then at 200° until excess aniline ceased to distill. A benzene extract of the residue on cooling deposited 1.86 g. of acetanilide m.p. 114–115°.

The crude borneol, after sublimation melted at 206–208° $[\alpha]_{D}$ −16.9° (c = 10.145 in toluene). The *p*-nitrobenzoyl ester of this substance was crystallized repeatedly from ethanol until melting point and rotation remained unchanged by further crystallization. It melted at 152–154°, $[\alpha]_{D}$ −0.8 (c = 10.05 in chloroform).

The ester was refluxed for one hour with excess 0.5 *N* alcoholic potassium hydroxide, and poured into water. After sublimation the recovered borneol melted at 201–206°, $[\alpha]_{D}$ −0.2° (c = 8.18 in toluene).

d-**Canadene.**—A mixture of 1.0 g. of fraction 83 (n_{D} 1.4949, d^{20}_{4} 0.926, α_{D} +26.79) and 0.10 g. of palladium-charcoal was heated under carbon dioxide for 6.5 hours, during which time the temperature was slowly raised from 200 to 243°. The reaction mixture was diluted with hexane, filtered and the filtrate extracted with 5% aqueous sodium hydroxide. The extracted filtrate was added to 10 ml. of 10% absolute ethanolic picric acid and refluxed for 15 minutes. On cooling 540 mg. of cadalene picrate separated, m.p. 116.5–117.5°, identified by comparison with an authentic sample. A solution of 270 mg. of fraction 83 in an equal volume of dry ether was treated with dry hydrogen chloride at −20°; 90 mg. of *l*-cadinene dihydrochloride m.p. 120–120.5°, $[\alpha]^{27}_{D}$ −39.3° (c = 6.37 in chloroform) was obtained.

Acknowledgments.—This investigation was undertaken at the suggestion of Mr. R. N. Johnston, Chief, Division of Research, Department of Lands and Forests, Province of Ontario, and his colleagues, to whom we are also indebted for supplies of plant material. The essential oil was prepared by Mr. J. N. Brown and Dr. T. F. West. The investigation was supported by a grant from the Research Council of Ontario.

TORONTO 5, CANADA RECEIVED NOVEMBER 9, 1950

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[Contribution from the Northern Regional Research Laboratory[1]]

Analysis of Fat Acid Oxidation Products by Countercurrent Distribution Methods. II. Methyl Oleate[2]

By J. Fugger,[3] K. T. Zilch, J. A. Cannon and H. J. Dutton

Samples of methyl oleate, oxidized at 37° to levels of 0.482 and 0.854 mole oxygen per mole ester have been fractionated between 80% ethanol and hexane with the use of countercurrent distribution methods. Three major components are apparent from the weight distribution curve: (1) unoxidized methyl oleate accounting for 62.6 and 41.96%, respectively, of the total weights; (2) a component consisting primarily of methyl oleate hydroperoxide (maximum purity 78%); and (3) a component consisting of peroxides, acidic scission products, and other oxidized substances. While the monohydroperoxide component is greater at the higher oxidation level, the increase in the third component relative to the second is greater still. The peroxide value in the third component remains approximately equal to that of the second, yet its hydrogen absorption value is greatly reduced indicating reaction of oxygen at the double bond. The evidence supports the view that monohydroperoxides are the first stable product in the reaction with gaseous oxygen and that the ethylenic bond is not attacked until a subsequent oxidation. No evidence of dimer formation was observed.

Except for recent studies, chemical characterization of oxidation products of methyl oleate have been performed either on unfractionated oxidation mixtures[4,5,6] or on single compounds isolated from the mixture.[7,8,9] By either approach, the concept of the over-all composition of the original reaction mixture has been obscured and has been responsible in part for two widely divergent schools of inter-

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(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946. Article not copyrighted.

(2) A portion of this paper is based upon a thesis to be submitted by J. Fugger in partial fulfillment of the requirements for the Degree of Doctor of Philosophy at the University of Pittsburgh. This work was carried out under an agreement between the University and the U. S. Department of Agriculture. Presented at 118th National Meeting of the American Chemical Society, September 3-8, 1950, in Chicago, Illinois.

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pretation—one, that primary reaction of gaseous oxygen is the saturation of the ethylenic bond,[10,11,12] the other, that the α-methylenic carbon is the site of formation of a hydroperoxide group.[6,13–16]

In recent studies, chromatographic adsorption analysis was applied to the resolution of oxidation mixtures.[7,17,18] It was found that despite the mildness of adsorption fractionation, as compared to usual techniques of fractionation, reaction and irreversible adsorptions do occur. These disadvantages which are inherent in adsorption analysis, however, are not present in solvent partition procedures. Application of the differential solubility principle to fractionation of oxidation mixtures has been greatly facilitated by the development of countercurrent distribution apparatus.[19,20]

This paper deals with the use of countercurrent distribution methods applied in a survey of the composition of oxidized methyl oleate. The study was undertaken as a necessary step to the understanding of oxidation in other and more complex oxidizing systems.

Materials and Methods.—A 29-tube preparative model of the Craig countercurrent distribution apparatus was used. Solvents employed were mutually saturated 80% ethanol and pentane–hexane (b.p. 37–56°).

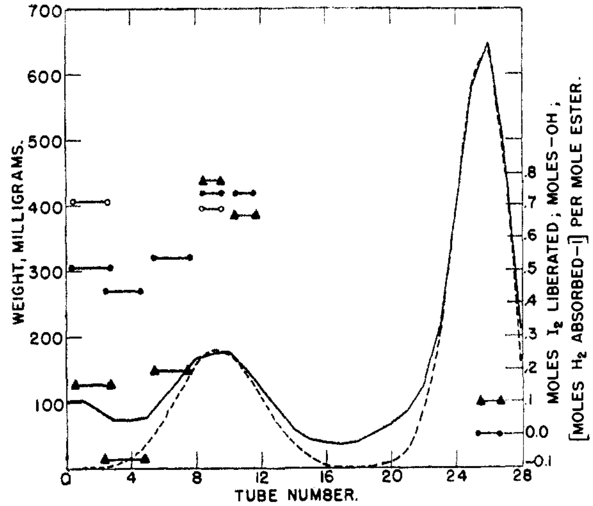

Fig. 1.—Weight and analysis of fractions of oxidized methyl oleate (0.482 mole oxygen absorbed per mole ester) after countercurrent distribution: ————, experimental weight curve; - - - - - -, theoretical weight curve; ●——●, moles I_2 liberated/mole ester; O——O, moles OH/mole ester; ▲——▲, [moles H_2 absorbed-1]/mole ester.

The analyses performed upon dissolved material of the individual tubes after countercurrent distribution include: (1) total weight, (2) hydrogen absorption, (3) peroxide value, (4) hydroxyl determination, (5) acid number and (6) molecular weight.

Hydrogen absorption was determined on a semimicro scale[21] using a palladium–barium sulfate catalyst.[22]

Peroxide values were determined by hydriodic acid reduction according to a procedure modeled after that of Lea, Bergström and Lundberg.[17,23,24]

The percentage of hydroxyl formed from the peroxides after hydrogenation of the oxidized esters was determined by a micro procedure developed in this Laboratory[25] involving the use of a phthalic anhydride–pyridine esterification reagent.[26]

Acid number determinations (mg. potassium hydroxide per g. sample) were made on material recovered after hydrogenating the peroxides.

Molecular weights were determined with use of diethyl ether as solvent by[27] the isothermal distillation procedure of Zigner.

Methyl oleate used for these experiments was obtained from the Hormel Institute, and had an iodine value of 85.43 (theory 85.62).

The oxidation of the methyl oleate was conducted at a temperature of 30 ± 2° on amounts of 2.5 and 5 g. Oxidation was speeded by the light of a mercury arc (G.E. H-4) placed at a distance of 20 cm. and was followed by means of manometric methods.

Results and Discussion

The distribution of weight with tube number for methyl oleate oxidized to a level of 0.482 mole oxygen per mole ester is plotted in Fig. 1. Three peaks are immediately apparent, corresponding to at least three major components. A comparison of the curve for the first component on the right with that of methyl oleate[28] permits the inference that the material comprising this curve is unoxidized methyl oleate. The second component whose maximum is in tube 9 would, by inference from the distribution curve for methyl ricinoleate, be attributed to the monohydroperoxide of methyl oleate.[28] The third component with maximum concentration in tubes 0 and 1 should be methyl oleate with two or more functional groups and acidic scission products of shorter chain length.[4,5,28] Chemical evidence supporting these inferences are given in Table I.

Reliance on results obtained by calculation of theoretical curves,[29] appears justifiable for these types and concentrations of materials because of the close agreement of calculated and experimental curves for model compounds distributed under comparable conditions.[28]

If one mole of stable hydroperoxide were formed per mole oxygen absorbed, 51.6% [(1.00−0.485) × 100] should have remained unoxidized. It is readily apparent by inspection of Fig. 1 that more than 51.6% of the methyl oleate remains unoxidized and therefore the level of oxidation of the oxidized portion must be greater than 1 mole per mole ester.

The oxygen content of the third peak can be estimated if the area under the monoperoxide theoreti-

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TABLE I

ANALYSES OF OXIDIZED METHYL OLEATE[a] AND FRACTIONS AFTER COUNTERCURRENT DISTRIBUTION

	Tube number 1-2	3-4	6-7	9	11	26	Oxidized methyl oleate
Peroxide value	3090	2670	3280	4490	4490	64	1860
Hydrogen no.	284	354	274	184	196	269	220
Hydroxyl, %	3.9			3.4			1.0
$\frac{\text{Mole peroxide}}{\text{Mole ester}}$	0.51	0.44	0.54	0.74	0.74	0.01	0.29
$\frac{\text{Mole } H_2 \text{ absorbed}}{\text{Mole ester}}$	1.16	0.93	1.20	1.78	1.67	1.10	1.42
$\frac{\text{Mole hydroxyl}}{\text{Mole ester}}$	0.71			0.69			
Acid no.	54.5			9.7			4.7
Mol. wt.	314			328		302	

[a] 0.482 mole oxygen absorbed per mole ester.

cal curve or the weight of monohydroperoxide multiplied by its assumed oxygen content (one mole of oxygen per mole of ester) is subtracted from the product of the total weight multiplied by 0.482 mole oxygen per mole ester and divided by the remaining weight of oxidized material (peak 3). Based on these assumptions the average oxygen content of the material on the left of the curve is 1.97 moles oxygen per mole ester.

Chemical structure of the oxidation products separated by solubility properties is clarified by the analytical work on the individual tubes given in Table I. Although the calculations of oxygen balance indicate an average of 2 moles of oxygen per mole of methyl oleate in tubes 0 to 4, the material in the tubes had a molecular weight of about 314 instead of 360 as would be the case if monomeric diperoxide were the only component. The comparatively large amount of acidic scission products in these tubes (determined after hydrogenation), accounts, no doubt, for the low molecular weight. Because of the experimentally determined molecular weights, it was thought more justifiable to use a molecular weight value of 328 to calculate all peroxide and hydrogen absorption ratios of tubes 0 to 12.

Ideally, methyl oleate hydroperoxide yields 1 mole peroxide oxygen (determined by hydriodic acid reduction), requires 2 moles of hydrogen for reduction (1 mole for the double bond and 1 mole for the hydroperoxide group) and after H_2 reduction, possesses 1 mole of hydroxyl per mole ester.

In tubes 9 and 11 the moles of peroxide oxygen per mole ester is in fair agreement with the moles hydrogen absorbed. From the shape of the distribution curve, considerable homogeneity and purity is indicated; however, hydrogen absorption data indicate only 0.78 and 0.67 mole of reducible peroxide per mole ester are present and hydriodic acid reduction data indicate only 0.738 mole. Based on these analyses, the maximum purity of the monoperoxide is 78%. The hydroxyl content after hydrogenation is 68.5% of that obtained for pure methyl hydroxystearate and supports the purity figures calculated from hydriodic acid reduction and hydrogenation.

The moles hydrogen absorbed and moles peroxide per mole ester diverge in the tubes numbered lower than 9 and indicate either the presence of structures which are reducible by hydrogen iodide but not by the palladium catalyst at room temperature or, more probable, the reaction of oxygen at the double bond to yield diketo, keto-hydroxy, dihydroxy, and oxirane groups. A slightly higher hydroxyl content after hydrogenation occurs in the lower numbered tubes than in those of the monoperoxide peak, confirming the more polar nature of materials in the lower numbered tubes. The hydroxyl content after hydrogenation is 71% of that obtained for pure methyl hydroxystearate.

Acidic oxidation products are apparently concentrated in the alcohol-soluble fractions of the first tubes. If these acids are calculated as monobasic C_9 acids of average molecular weight 180, they will account for 18% of the weight of tubes 1 and 2. Postulation of monobasic C_9 split products is compatible with the lower molecular weight figure obtained for the first tubes. In fact, if one assumes

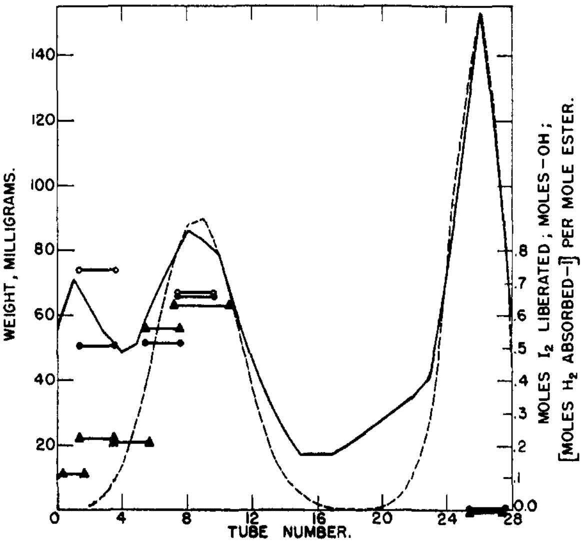

Fig. 2.— Weight and analysis of fractions of oxidized methyl oleate (0.854 mole oxygen absorbed per mole ester) after countercurrent distribution; ————, experimental weight curve; - - - - - -, theoretical weight curve; ●———●, moles I_2 liberated/mole ester; ○———○; moles OH/mole ester; ▲———▲, [moles H_2 absorbed-1]/mole ester.

TABLE II

ANALYSES OF OXIDIZED METHYL OLEATE[a] FRACTIONS AFTER COUNTERCURRENT DISTRIBUTION

	Tube number 0	1	2-3	4-5	6-7	8-9	10	11-12	26-27
Peroxide value	3500		3070		3140	4010			140
Hydrogen no.		297	269	279	211	202	202	197	300
Hydroxyl, %			4.0			3.6			
$\frac{\text{Mole peroxide}}{\text{Mole ester}}$	0.58		0.51		0.52	0.66			0.02
$\frac{\text{Mole H}_2\text{ absorbed}}{\text{Mole ester}}$		1.11	1.22	1.18	1.56	1.63	1.63	1.67	0.99
$\frac{\text{Mole hydroxyl}}{\text{Mole ester}}$			0.74			0.67			
Acid no.			56.8			17.6			
Mol. wt.		318	324			322	327		303

[a] 0.854 mole oxygen absorbed per mole ester. Peroxide value of original sample = 2520 or 0.408 mole peroxide/mole methyl oleate.

that the lower numbered tubes are made up of a mixture of monomeric diperoxide and C_9 acids, 23% by weight of the acids would account for the lowering of the molecular weight of the material in these tubes.

The molecular weight determinations are of particular interest in that they give no evidence of polymer formation in the oxidation of methyl oleate.

Another oxidation and countercurrent distribution experiment is presented in Fig. 2 (and in Table II). It was similar to the one just described except that the oxidation was carried to 0.854 mole oxygen per mole ester. The results obtained are in general agreement. More of the ester was, of course, found to be oxidized. The divergence between weight of the oxidized fraction predicted on a simple monoperoxide basis and the actual weight was greater than before; whereas, 85.4% is calculated to have been oxidized on a simple monoperoxide reaction basis, only 58.0% was found to be oxidized experimentally. The average level of oxidation of the low number tubes calculated in a manner similar to that described above was 2.45 moles oxygen per mole ester.

Chief differences between the results of these experiments came in the increased amounts of material in the oxidized parts of the curve, particularly the peak in tube no. 1. The evidence that the third component is relatively increased over the second at the higher oxidation level is interpreted as showing that after the monohydroperoxide is formed, the subsequent attack of oxygen in either gaseous or peroxidic form is at the ethylenic bond.

Acknowledgment.—The authors are grateful to J. C. Cowan for his interest and encouragement throughout the course of the work.

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[CONTRIBUTION FROM THE PHARMACEUTICAL RESEARCH SECTION, CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Analogs of Pteroylglutamic Acid. VII. 2-Alkylamino Derivatives

BY BARBARA ROTH, JAMES M. SMITH, JR., AND MARTIN E. HULTQUIST

2-Alkylamino analogs of pteroylglutamic acid and its 4-amino derivative have been synthesized by the reaction of the appropriate 5,6-diaminopyrimidines with 2,3-dibromopropanal and *p*-aminobenzoylglutamic acid. A number of new 2-alkylaminopyrimidines and pteridines are described.

The synthesis of a number of analogs of pteroylglutamic acid (I)[1] has been described in earlier communications from this Laboratory,[2] and since some of these are potent antagonists[3] for I it seemed advisable to investigate further modifications in the molecule.

The toxicity of N-[4-(2,4-diamino-6-pteridylmethyl)-aminobenzoyl]-glutamic acid (II) ("Aminopterin," "4-aminopteroylglutamic acid") was greatly reduced by the substitution of alkyl radicals on the 4-amino group.[4] The present paper describes the preparation of some analogs of I, and other pteridines, substituted in the 2-position by alkylamino groups.

2-Dimethylaminopyrimidines were synthesized by the condensation of 1,1-dimethylguanidine with cyanoacetic ester and malononitrile, to give 2-dimethylamino-4-hydroxy-6-aminopyrimidine and 2-dimethylamino-4,6-diaminopyrimidine, respectively. These compounds were readily nitrosated in the 5-position and reduced to 5,6-diaminopyrimidines which reacted with diacetyl to produce 2-dimethylamino-4-hydroxy- and 2-dimethylamino-

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