

Methods for the Isolation and Characterization of Constituents of Natural Products

A Periodic Acid Column Procedure for the Oxidation of Vic-Glycols, Epoxides, and α -Hydroxy Acids at the Micromole Level

D. P. Schwartz, J. L. Weihrauch, and L. H. Burgwald¹

Dairy Products Laboratory, Eastern Utilization Research and Development Division,² Washington, D. C. 20250

THE UTILIZATION of periodic acid and its salts in organic structural studies, in certain syntheses, and in organic analyses has been thoroughly documented in a number of reviews (1-3). In an attempt to expand the scope of the periodic acid reaction and make it more suitable for the oxidation of susceptible structures in lipids, a two-phase oxidation procedure has been evolved in which the polar phase (aqueous periodic acid) is impregnated onto a support (calcium sulfate) and a water-immiscible solvent containing the substrate is passed over the support. The product(s) of the reaction then emerge at the exit end of the column.

EXPERIMENTAL

Reagents and Apparatus. Calcium sulfate, anhydrous powder, was obtained from the J. T. Baker Co., Phillipsburg, N. J.; paraperiodic acid (H_5IO_6) was a product of the G. Frederick Smith Co., Columbus, Ohio; methylene chloride (Baker) was used as received; benzene, ACS grade (thiophene-free) (Baker) was rendered carbonyl-free as described by Schwartz and Parks (4) then distilled from glass; *n*-hexane, High purity grade (Phillips Petroleum Co., Bartlesville, Okla.) was purified by the method of Hornstein and Crowe (5) but using a greatly reduced flow rate—*i.e.*, approximately one liter/24 hours than was recommended in that paper. The purified hexane was then distilled from glass; aluminum oxide, Brockman activity grade I, acidic, for chromatography (Baker) was partially deactivated by the addition of 8% distilled water. The substrates employed in the study are listed in Table I along with their source and estimated purity.

Procedure. PREPARATION OF PERIODIC ACID COLUMNS. One milliliter of a saturated solution of periodic acid in distilled water is pipetted into a 4-inch mortar and ground with 5 grams of $CaSO_4$ until the powder appears homogeneous. This entails grinding, scraping the mortar with a flat spatula, and regrinding. This operation is repeated several times. The powder is then stored in a suitable receptacle with a secure lid. The powder has been stored for up to 6 months at room temperature with no apparent ill-effects.

Columns are prepared by transferring 0.5 gram of the powder to a chromatography tube approximately 0.8×15 cm. The powder is tamped lightly with a tamping rod so that after the column is wetted with hexane, 1 ml of this solvent will flow completely into the column in 9 to 11 minutes. If the

flow rate exceeds that, the column should be tamped tighter at this point until the desired flow rate is attained. With a little experience, columns can be prepared with very little variation in flow rate. The column is then washed with a column volume of benzene and is ready for the sample. The periodic acid columns prepared as described contain over 300 μ moles of periodic acid and can be used repeatedly. The entire study was done with two such columns.

PREPARATION OF 2,4-DINITROPHENYLHYDRAZINE COLUMN. The extent of the periodic acid reaction was followed by permitting the effluent from the $CaSO_4$ column to drip directly onto a column of Celite impregnated with a phosphoric acid solution of 2,4-dinitrophenylhydrazine (4). The carbonyl fragments are immediately derivatized and subsequently analyzed. Columns are prepared by transferring 2.5 grams of the impregnated Celite to a column (1.7 cm i.d. \times 17 cm) and tamping fairly tightly so that the flow rate of hexane through the column will be between 40-50 ml/hr. The column is washed with 2 column volumes of benzene and then with hexane until the effluent emerges colorless. Celite impregnated with the 2,4-dinitrophenylhydrazine solution can be stored at $-18^\circ C$ and used as needed.

PREPARATION OF SUBSTRATE SOLUTIONS. Solutions of the glycols, epoxides, and α -hydroxy acids listed in Table I were made in benzene except for lactic and mandelic acids and 1,2-propanediol which were made in methylene chloride which is pure as far as carbonyl-contaminants are concerned (4). Solutions of the substrates were prepared to contain between 0.8 to 4.0 μ moles/ml. The concentrations of the substrate employed are listed in Table I.

OXIDATION AND DERIVATIZATION PROCEDURE. The periodic acid column is positioned in the 2,4-dinitrophenylhydrazine column so that the tip of the former is just above the top of the bed. An aliquot of the substrate solution (0.25 to 1.0 ml) is pipetted onto the periodic acid column and when drained the sides of the column are washed with 1 ml of benzene. When this has drained the periodic acid column is washed with 1 ml more of benzene which completes the removal of the products of oxidation. All washings are permitted to drain into the bed of dinitrophenylhydrazine and 5 ml of benzene is added. When this has percolated into the column, hexane is added until the effluent emerges colorless.

ISOLATION, ESTIMATION, AND IDENTIFICATION OF DERIVATIVES. The effluent is mixed and transferred to a 5-gram column of alumina contained in a chromatographic tube approximately $0.8 \text{ cm} \times 15 \text{ cm}$. The simple aliphatic carbonyls are eluted with (1:1) hexane:benzene; aromatic and poly-functional carbonyls are removed with benzene. In any case, when dealing with unknowns, elution should be first attempted with benzene:hexane (1:1) for this also gives some clue regarding the nature of the derivative.

The solvent is evaporated and the optical density of the residue dissolved in $CHCl_3$ is determined at the wavelength of maximum absorption (Table I). Concentrations were calculated using the molar absorptivities listed in Table I.

The derivatives were spotted on thin-layer partition plates

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² Agricultural Research Service, U. S. Department of Agriculture.

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Table I. Periodic Acid Column Oxidation of Glycols, Epoxides, and α -Hydroxy Acids

Substrate	Source	Estimated purity, %	Amount of substrate employed, μ moles	Average yield, %	Identity of 2,4-dinitrophenylhydrazones	γ Max CHCl_3 used	$E \times 10^{-3}$ used
Benzoin	Aldrich ^a	...	0.5-2.0	100	Benzaldehyde	378	28
Benzopinacol	Aldrich	...	0.25-1.0	0
Threo-9,10-dihydroxymethyl stearate	EURDD ^b	95	0.2-0.8	99	Nonanal, methyl azelaldehydate	355	22.5
DL-1,2-Diphenyl-1,2-ethanediol	Schuchardt ^c	...	0.25-1.0	102	Benzaldehyde	378	28
Meso-1,2-diphenyl-1,2-ethanediol	Schuchardt	...	0.35-1.4	103	Benzaldehyde	378	28
9,10-Epiminooctadecane	EURDD	98.5	0.25-1.0	0
1,2-Epoxy butane	Aldrich	99 ^b	1.0-4.0	79	Propanal	355	22.5
1,2-Epoxy octane	EURDD	95 ^b	0.6-2.4	90	Heptanal	355	22.5
9,10-Epoxy methyl stearate	EURDD	95	0.45-1.8	63	Nonanal, methyl azelaldehydate	355	22.5
Glyceryl-1-hexadecyl ether	Fluka ^d	puriss	0.8-3.2	93	Hexadecoxy acetaldehyde	355	22.5
Glyceryl-1-octadecyl ether	Fluka	puriss	0.75-3.0	95	Octadecoxy acetaldehyde	355	22.5
α -Hydroxy lauric acid	Aldrich	...	0.5-2.0	72	Undecanal	355	22.5
2-Hydroxy-2-methyl 3-butanone	Aldrich	87 ^b	0.75-3.0	100	Acetone	362	22.5
2-Hydroxy-2-methyl butyric acid	Aldrich	...	0.5-2.0	73	2-Butanone	362	22.5
α -Hydroxy-methyl eicosanoate	Applied Science ^e	99	0.4-1.6	0
α -Hydroxy-myristic acid	Aldrich	...	0.5-2.0	74	Tridecanal	355	22.5
DL-Lactic acid	Lachat ^f	...	0.75-3.0	38	Acetaldehyde	362	22.5
DL-Mandelic acid	Aldrich	...	0.5-2.0	102	Benzaldehyde	378	28
Methyl mandelate	Aldrich	...	1.0-4.0	0
1-Monopalmitin	Supelco ^g	99	0.5-2.0	95	Glycolaldehyde palmitate	350	20
1-Monostearin	Supelco	99	0.5-2.0	91	Glycolaldehyde stearate	350	20
1-Phenyl-1,2-ethanediol	Aldrich	...	1.0-4.0	105	Benzaldehyde	378	28
Pinacol	Aldrich	98	0.2-0.8	87	Acetone	362	22.5
1,2-Propanediol	Aldrich	...	1.0-4.0	56	Acetaldehyde	355	22.5
<i>trans</i> -Stilbene oxide	Aldrich	...	0.7-2.8	83	Benzaldehyde	378	28
Styrene oxide	Aldrich	...	0.75-3.0	97	Benzaldehyde	378	28

^a Aldrich Chemical Co., Milwaukee, Wis.

^b Eastern Utilization Research and Development Division, ARS, USDA, Philadelphia, Pa.

^c Schuchardt Chemische Fabrik, Munich, Germany.

^d Fluka Ag., Chemische Fabrik, Buchs, Switzerland.

^e Applied Science Laboratories, Inc., State College, Pa.

^f Lachat Chemicals, Inc., Chicago, Ill.

^g Supelco, Inc., Bellefonte, Pa.

^h By vapor phase chromatography 50' \times 3/8" SE-30 column 240-280 °C.

along with the expected authentic derivatives. The neutral and alkaline partition systems described by Schwartz *et al.*, (6) were utilized.

RESULTS AND DISCUSSION

Pertinent data are presented in Table I. Yields are the average of four levels of substrate (2, 3, and 4 times the lowest concentration) analyzed and are based on the estimated purity of the substrate when known, or on 100% purity if unknown.

Yields were, for the most part, very good for vic-glycols with the exception of 1,2-propanediol and for pinacol. Benzopinacol, a highly hindered vic-glycol, did not oxidize at all.

Epoxy compounds with the exception of styrene oxide failed to oxidize quantitatively. Maerker and Haerberer (7)

could get quantitative yields on only one epoxide out of four which they subjected to periodic acid oxidation in dioxane-water mixtures.

The α -hydroxy acids investigated unexpectedly were oxidized and in fair yields. This structure is not readily attacked by periodic acid in solution even at elevated temperatures (1, 8, 9), although both lead tetraacetate and sodium bismuthate attack that structure (10). Mandelic acid yielded the theoretical amount of benzaldehyde. The aliphatic α -hydroxy acids, however, gave lower yields. The methyl esters of α -hydroxy acids as expected, did not undergo oxidation.

In connection with yields, it should be pointed out that one set of conditions only, chosen arbitrarily at the outset, was used. No attempt was made to improve yields in those instances where quantitative yields were not obtained. Consequently, it is possible that improved yields could be obtained

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by the appropriate variation of parameters, for example, by changing flow rates, solvent, raising the temperature, adjusting the pH, manipulating the periodic acid concentration, or by incorporating salt into the aqueous phase.

An interesting observation made during this study was the complete absence of formaldehyde in the products of oxidized substrates in which formaldehyde is predicted. No trace of the 2,4-dinitrophenylhydrazone of formaldehyde was evident on the thin layer plates. Even when 100 mg of 1-monopalmitin or glycerin-1-octadecylether were oxidized formaldehyde was not detected by odor or by analysis of the 2,4-dinitrophenylhydrazones. The fate of the formaldehyde was not determined.

Periodic acid-impregnated Celite and fine glass beads were unsatisfactory supports for the oxidation.

The most obvious advantage of employing the periodic acid column for the oxidation of susceptible structures in lipids is that the need for finding a suitable mutual solvent is circumvented. Maerker and Haerberer (7) point out that the ratio of water to dioxane which they used as a solvent in their studies

was fairly critical as far as yields and solubility of the substrate were concerned.

The periodic acid column procedure would seem to be more amenable than a one-phase system for structural studies on microgram quantities of an organic compound. The columns can be reduced in size which accordingly will decrease the volumes involved. Thus analysis of the effluent by gas-liquid chromatography-mass spectrometry, for example, should be possible without further manipulations such as extraction or evaporation.

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