

Methods for the Isolation and Characterization of Constituents of Natural Products

XII. A Chromic Acid Column Procedure for the Quantitative Oxidation of Secondary Alcohols at the Micromole Level

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In the course of an investigation on the hydroxylated constituents of lipids, a simple method was needed to oxidize trace amounts of secondary alcohol structures which occur in lipids to the corresponding ketone in order to locate the position of the hydroxyl group via the Beckmann rearrangement. It was found that rapid and thorough oxidation could be conveniently achieved on microamounts of secondary alcohols by passing a CCl_4 solution of the alcohol over a column of Celite impregnated with aqueous chromic acid.

Chromic acid oxidation of alcohols has been extensively studied (1, 2) 6-8) and although an array of solvent systems including liquid-liquid and solid-liquid systems have been proposed, the use of a column of the oxidant has apparently not been exploited.

MATERIALS¹

Carbon tetrachloride (Baker Chemical Co., Phillipsburg, N.J., cat. no. 1512) was used as received. It was analyzed for carbonyl compounds and alcohols and found to be completely free of these classes. Benzene (Baker) and *n*-hexane (Phillips High Purity Grade, Phillips Petroleum Co., Bartlesville, Oklahoma) were rendered carbonyl-free by the procedure of Schwartz and Parks (4); alumina, acidic, Brockmann Activity Grade I (Baker) was partially deactivated by the addition of 8% distilled water. The alcohols (Table 1) were obtained from various commercial and private sources. The methylhydroxystearates were kindly supplied by Dr. A. P. Tulloch, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada; chromic anhydride was obtained from the Fisher Scientific Com-

¹ Mention of brand or firm name does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

pany, Silver Spring, Maryland; Analytical Grade Celite was a product of the Johns-Manville Co., Baltimore, Maryland.

EXPERIMENTAL METHOD

Preparation of chromic acid column. Two ml of a 50% solution of chromic anhydride in distilled water was ground in a 4-inch mortar with 4 g of Analytical Grade Celite until the Celite was homogeneously yellow. The powder, which contains about 1.7 μ moles of chromic acid/mg of impregnated Celite, was stored at room temperature in a vial with a secure lid.

To prepare a chromic acid column, 100 mg of the impregnated Celite was placed in a medicine dropper (approx. 0.5 \times 9.5 cm) containing a glass wool plug at the tapered end. The dropper was tapped several times on the bench top to pack the Celite. Columns prepared in this manner have flow rates averaging about 8 ml/hour of CCl_4 . Any flow rate between 6 and 10 ml/hour is satisfactory. The column will turn dark from the top down as its oxidizing ability is exhausted. This is readily seen and can be used as an indicator of the remaining oxidizing potential of the column. When anticipating the use of a column, it should be washed with 2–3 column volumes of CCl_4 prior to addition of the alcohol solution.

Preparation of 2,4-dinitrophenylhydrazine column. The extent of the chromic acid oxidation was followed by permitting the effluent from the chromic acid column to enter a column of Celite impregnated with a phosphoric acid solution of 2,4-dinitrophenylhydrazine (4). The carbonyls in the effluent are derivatized, subsequently purified and estimated spectrophotometrically.

The impregnated Celite is prepared as described by Schwartz and Parks (4). A column of the impregnated Celite is made by dry packing 30 g of the impregnated Celite into a chromatography tube 2.5 cm i.d. \times 30 cm. The Celite is transferred to the tube in 6–8 portions, tamping fairly tightly between portions so that the flow rate of CCl_4 through the column will be between 40 and 50 ml/hour. The column is washed with 1 column volume of carbonyl-free benzene followed by 1–2 column volumes of CCl_4 and is then ready for use. The washing procedure is conducted each day the column is to be used.

Oxidation and derivatization procedure. The chromic acid column is positioned in the 2,4-dinitrophenylhydrazine column so that its tip is just above the bed. A volume (up to 2 ml) of a CCl_4 solution of the alcohol is pipetted into the chromic acid column and when this has drained, the sides of the medicine dropper are washed down with about 0.25 ml of CCl_4 ; when this has drained about 0.5 ml of CCl_4 is added to complete

TABLE 1
OXIDATION OF ALCOHOLS BY CHROMIC ACID ON A CELITE COLUMN

Alcohol	Source	Estimated purity ^c (%)	Amount used (μ moles)	Yield(%)
3 β -Cholesterol	M ^a	—	2.2	103
Cyclohexanol	M	99	1.0	111
			2.0	109
3-Decanol	A ^b	100	2.0	97
2,2-Dimethyl-3-heptanol	A	100	2.0	100 ^d
2-Heptanol	A	96	2.0	97
3-Hexanol	M	100	0.5	107
			2.0	101
L-Menthol	A	100	0.5	104
			2.0	95
2-Methyl-3-heptanol	A	—	2.0	100
4-Methyl-3-heptanol	A	—	2.0	86 ^e
			1.0	87 ^e
2-Methyl-3-hexanol	A	—	2.0	98
3-Methyl-2-hexanol	A	—	2.0	98
Methyl-2-hydroxystearate	A		2.1	68
Methyl-6-hydroxystearate	See text		1.9	97
Methyl-7-hydroxystearate	See text		2.2	92
Methyl-12- <i>d</i> (-)-hydroxystearate	See text		1.9	98
Methyl-13-hydroxystearate	See text		2.3	103
Methyl-17-hydroxystearate	See text		2.6	94
Methyl-18-hydroxystearate	See text		1.7	55
2-Methyl-4-nonanol	A	99	2.0	103
2-Methyl-3-nonanol	A		2.0	98
3-Methyl-4-nonanol	A		1.0	69
2-Methyl-3-octanol	A		2.0	91
3-Methyl-4-octanol	A		1.0	91 ^e
3-Methyl-2-pentanol	A	100	2.0	99
2-Methyl-3-pentanol	A	100	2.0	96
4-Methyl-2-pentanol	A	99	2.0	100
2-Nonadecanol	A		2.2	102
2-Nonanol	A	94	2.1	95
5-Nonanol	A	100	2.0	94
			0.5	100
4-Octanol	M	100	2.0	98
			0.5	104

^aMatheson, Coleman and Bell, Inc., East Rutherford, N. J.

^bAldrich Chemical Co., Milwaukee, Wis.

^cPurity estimated by gas-liquid chromatography.

^d2,2-Dimethyl-3-heptanone did not react at all with 2,4-dinitrophenylhydrazine on the column. The yield was estimated by GLC using authentic 2,2-dimethyl-3-heptanone as standard.

^eAuthentic ketones corresponding to the expected oxidation product of these alcohols reacted sluggishly on the 2,4-dinitrophenylhydrazine column. This probably accounts for the less than quantitative yields.

the flushing out of the chromic acid column. When all of the washings have entered the bed of 2,4-dinitrophenylhydrazine, it is washed with sufficient CCl_4 to remove the derivatives. This is signaled by a diminution of color in the effluent. Unless the carbonyl derivative is very polar, two column volumes will remove all of the derivative.

Isolation and estimation of derivatives. The effluent from the 2,4-dinitrophenylhydrazine column is transferred to a column of alumina (5 g poured dry into a chromatography tube 0.7×20 cm) and the derivative is eluted with benzene:hexane (1:1) until all color is removed from the bottom portion of the column. The solvent is evaporated to dryness and the residue read in CHCl_3 at $363 \text{ m}\mu$. Concentration of carbonyl is estimated from the reading using $E = 22,500$.

Thin-layer partition chromatography. The derivatives obtained from the alumina column were examined for purity using the neutral and alkaline thin-layer partition systems described by Schwartz *et al.* (5). Authentic derivatives, when available, were spotted on adjacent spots and the plate developed with *n*-hexane saturated with polyethylene glycol 400.

Assay of alcohols. When yields of carbonyl were less than quantitative, an effort was made to determine whether oxidation had been complete or whether some other factor was responsible. This was accomplished by running an aliquot of the alcohol solution over the chromic acid column and assaying the effluent directly for alcohol with pyruvic acid chloride 2,6-dinitrophenylhydrazone (3).

RESULTS AND DISCUSSION

Table 1 presents the data pertaining to the study. Yields are based on the purity as estimated by gas-liquid chromatography or on 100% if the purity was unknown. Table 1 reveals that very good yields of ketones were obtained by oxidizing a variety of secondary alcohols by the chromic acid column procedure. Analyses for unoxidized alcohol (in those instances where less than about 95% yield was obtained) were completely negative suggesting that either impurities other than alcohols were present in the original sample when weighed, or side reactions had taken place, or, as was found in a number of instances, complete derivatization of the carbonyl resulting from the oxidation had not occurred on the 2,4-dinitrophenylhydrazine column. This was found to be the case with 4-methyl-3-heptanone, 3-methyl-4-nonanone, 3-methyl-4-octanone, and 2-methyl-3-octanone. In one instance (2,2-dimethyl-3-heptanone) no yield of 2,4-dinitrophenylhydrazone was obtained on the column although this ketone reacted slowly with 2,4-dinitrophenylhydrazine in ethanol solution. Assay of the ketone in the effluent from the chromic

acid column by gas-liquid chromatography showed that the alcohol had oxidized quantitatively to the ketone.

The oxidation of primary alcohols by the chromic acid column was complete on the primary alcohols that were tested. However, the yields of aldehydes were relatively low, methyl-18-hydroxystearate giving a 55% yield, other aldehydes (not listed) being even lower. Thus, even the relatively short residence time of a primary alcohol molecule on the two-phase column still results in further oxidation of the aldehyde to the acid.

Indications are that the oxidation of unsaturated alcohols on the chromic acid column gives rise to a number of side reactions resulting in the appearance of carbonyl fragments. Further work on faster columns at room temperature and below is anticipated in this regard and also for the oxidation of primary alcohols.

Thin-layer partition chromatography of the derivatives gave a single yellow spot except in a few cases where 2 spots were observed. In the latter instances, the original alcohol was accordingly investigated by temperature programmed gas-liquid chromatography on a fairly long (14 foot) column of Triton X 305 on 60-80 mesh AW-DCMS Chromosorb W and this usually revealed two partially separated peaks or an asymmetric peak suggesting the presence of isomeric compounds.

Although the smallest amount of alcohol oxidized by the chromic acid column is listed as approximately 0.5 μ mole, oxidations were performed on 0.1-0.2 μ moles with good but sometimes erratic yields of the carbonyl derivative. There should be no reason why qualitatively, at least, even smaller amounts of alcohols could not be oxidized on this size or on even smaller columns.

SUMMARY

A two-phase column procedure for the quantitative oxidation of saturated secondary alcohols to the corresponding ketone is described. Aqueous chromic acid on a column of Celite and a CCl_4 solution of the alcohol comprise the system. The method is well-suited for the oxidation of secondary alcohols at the micro- and even submicromole level.

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