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Methods for the Isolation and Characterization of Constituents of Natural Products

XVIII. A Micro-bisulfite Column for Extracting Aldehydes and Certain Ketones

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This paper reports the preparation and use of a micro-column of sodium bisulfite to remove certain carbonyl compounds from solution. The method was primarily developed for use as a step in the simplification of complex mixtures so that chromatograms could be interpreted more readily and missing peaks or spots could be tentatively classified.

A two-phase column procedure, similar in principle, previously had been reported by Parks, Keeney and Schwartz (*7, 8*) who used it in their study of fatty aldehydes liberated from the neutral plasmalogen fraction of milk fat. Their columns (30 g) were a disadvantage for work with small volumes. We have modified their procedure for use as a micromethod and have obtained satisfactory retention of certain carbonyl compounds using columns 1/150th the size for quantitative studies at the micromole level. The procedure also has been reduced to a point where it can be conducted in a melting point capillary on microgram amounts of carbonyls. The scope of the method has been expanded to include a number of types of carbonyl compounds not studied in the original method.

Except for the work of Parks, Keeney and Schwartz (*7, 8*), use of sodium bisulfite adduction of carbonyl compounds on columns apparently has been confined to anion-exchange resins in the bisulfite form (*1–4*). The ion-exchange methods, however, were applied only to water-soluble or water-alcohol-soluble carbonyl compounds and were on a semimacro scale.

MATERIALS AND EQUIPMENT[1]

Sodium bisulfite (Cat. No. 3556), CCl_4 (Cat. No. 1512) and benzene (Cat. No. 9154) were obtained from the J. T. Baker Chem. Co.,

[1] Mention of specific commercial products does not imply endorsement.

Phillipsburg, N. J.; *n*-hexane (high-purity grade) a product of the Phillips Petroleum Co., Bartlesville, Okla., was purified by the method of Hornstein and Crowe (*5*). Analytical Grade Celite (Fisher Sci. Co., Silver Spring, Md.) was dried at least 24 hr at 100°C. The carbonyl compounds were purchased mainly from the Aldrich Chemical Co., Milwaukee, Wis., or Eastman Kodak Co., Rochester, N. Y., or were generated as needed by passing the 2,4-dinitrophenylhydrazone over a periodic acid column (*9*). Octadecoxyacetaldehyde and 2-hexadecanoyloxyacetaldehyde were prepared by passing batyl alcohol and 1-monopalmitin, respectively, over a periodic acid column (*10*). Disposable Pasteur pipettes (145 × 7 mm o.d.) were used for columns. They were cut just below the crimp to facilitate insertion of the column materials and were plugged with a small wad of glass wool. For experiments at the microgram level, columns were made from melting point capillaries, 100 mm long 1.5–2.0 mm o.d. (Fisher Sci. Co., Silver Spring, Md., Cat. No. 12-141), which were cut approximately in half.

EXPERIMENTAL METHODS

Celite (1.4 g) and 1 ml of a freshly prepared saturated solution of sodium bisulfite in distilled water are ground in a mortar until homogeneous. The impregnated Celite should be prepared each day and stored at 0°C when not in use.

Construction and operation of column. For preparation of a column, 200 mg of impregnated Celite is transferred to a Pasteur pipette. The column is tamped with medium pressure so that 0.5 ml of hexane will enter the column (which had just previously been washed with 2 column volumes of hexane) in 7–8 min.[2] A solution of the carbonyl compound in a suitable solvent (see Table 1) or a mixture of carbonyl and noncarbonyl compounds containing up to 15 μmoles of the carbonyl compound[3] is pipetted onto the column. In this study 0.5 ml of the solution was used routinely, but there is no obvious reason why larger volumes cannot be used as long as the proper flow rate is maintained. When the solution has entered the bed completely, the sides of the column are washed down with a little solvent and, when this has drained, a column volume of solvent is added to wash out any remaining compounds not forming a bisulfite adduct.

[2] At a flow rate of 7–8 min/0.5 ml, most normal chain aliphatic and aromatic aldehydes can be extracted quantitatively. By decreasing the flow rate some carbonyl compounds not extracted quantitatively or at all at a flow rate of 7–8 min/0.5 m., can be extracted to various degrees (Table I).

[3] A freshly prepared column will hold at least 15 μmoles of carbonyl compounds. This value was established for palmitaldehyde. The upper limit of the column was not ascertained.

Quantitative aspects. To assess the efficiency of the bisulfite column for removal of carbonyl compounds, the effluent was passed over a 1 g column of 2,4-dinitrophenylhydrazine (DNP) on Celite (*11*) packed in a Pasteur pipette and positioned below the bisulfite column so that the tip of the latter rested on the bed of 2,4-dinitrophenylhydrazine. Following completion of washing of the bisulfite

TABLE 1
EXTRACTION OF VARIOUS CARBONYL COMPOUNDS BY A SODIUM BISULFITE–CELITE COLUMN

Carbonyl	Amount over column (μmoles)	Flow rate (min/0.5 ml)	Solvent	Retained (%)	Recovered (μmoles %)
Aldehydes					
Acrolein	0.47	7–8	Hexane	100	
Benzaldehyde	1.17	7–8	Hexane	100	1.24(105)
Isobutyraldehyde	0.50	7–8	Hexane	86	
Cinnamaldehyde	1.16	7–8	Hexane	96	
Crotonaldehyde	2.30	7–8	Hexane	100	
3,4-Dimethoxybenzaldehyde	0.50	7–8	Hexane	100	0.47(94)
2-Ethylbutyraldehyde	0.77	7–8	CCl_4	Trace	
	0.77	28–29	CCl_4	10	
2-Ethylhexanal	3.00	7–8	CCl_4	0	
	3.00	23–24		0	
2-Ethyl-2-hexenal	2.30	7–8	CCl_4	0	
	2.30	23–24	CCl_4	0	
2-Ethyl-isovaleraldehyde	1.78	7–8	CCl_4	0	
	1.78	19–20	CCl_4	0	
Furfural	0.57	7–8	CCl_4	100	0
2-Hexadecanoyloxyacetaldehyde	0.40	7–8	CCl_4	100	
2,4-Hexadienal	1.77	7–8	Hexane	100	0
Methyl-8-formyloctanoate	2.10	8–9	Hexane	100	
5-Methylfurfural	2.20	7–8	Hexane	100	
2-Methylundecanal	0.70	7–8	Hexane	0	
	0.70	22–23	Hexane	4	
Nonanal	3.90	7–8	Hexane	96	
Octadecoxyacetaldehyde	1.00	7–8	CCl_4	100	
Phenylacetaldehyde	1.25	8–9	Hexane	100	
Piperonal	1.20	7–8	Hexane	Trace[a]	
	1.20	45–46	Hexane	100[a]	
Stearaldehyde	0.64	7–8	Hexane	100	0.54(84)
Salicylaldehyde	1.65	9–10	CCl_4	100[a]	Trace
Tetradecanal	2.09	7–8	CCl_4	95	1.49(75)
Valeraldehyde	1.31	7–8	Hexane	100	
Isovaleraldehyde	3.78	9–10	Hexane	100	
Vanillin	1.70	11–12	Benzene	99[a]	0

[a] By gas–liquid chromatography of column effluent.

TABLE 1 (*Continued*)

Carbonyl	Amount over column (μmoles)	Flow rate (min/0.5 ml)	Solvent	Retained (%)	Recovered (μmoles %)
Ketones					
Acetone	1.35	7–8	Hexane	100	0.70(52)
2,3-Butanedione	3.30	8–9	Hexane	100[a]	
2-Butanone	1.11	7–8	Hexane	10	
	1.11	27–28	Hexane	100	0.55(50)
3-Cholestanone	1.80	7–8	CCl_4	0	
	1.80	23–24	CCl_4	0	
Cyclododecanone	2.10	7–8	CCl_4	0	
	2.10	27–28	CCl_4	0	
Cycloheptanone	4.40	12–13	CCl_4	0	
Cyclohexanone	2.32	9–10	Hexane	100	2.37(101)
Cyclopentanone	3.34	7–8	Hexane	100	
2,3-Heptanedione	3.66	13–14	Hexane	100[a]	
Methyl propyl ketone	1.04	7–8	Hexane	0	
	1.04	34–35	Hexane	Trace	
Methyl-β-ketoeicosanoate	1.52	43–44	Hexane	0	
Methyl-12-ketostearate	2.27	40–41	Hexane	0	
Methyl nonyl ketone	2.13	30–31	Hexane	0	

column, 2 column volumes of CCl_4 were added to the DNP column to elute the hydrazones which were then purified on alumina (*11*) and determined spectrophotometrically.

In a few instances, where it was not convenient or feasible to assay colorimetrically (see Table 1), the effluent from the bisulfite column was evaporated just to dryness at room temperature under a stream of nitrogen. The residue was taken up in 0.1 ml of CH_2Cl_2 and 4 μl was analyzed by gas–liquid chromatography under the following conditions: instrument, Hewlett-Packard, Model 5750; column, 4 ft. $\times \frac{1}{8}$ in. stainless steel packed with 7.5% ethylene glycol adipate plus 2% H_3PO_4 on 90–100 mesh Anakrom ABS; detector, flame ionization; carrier gas, helium at a flow rate of 30–35 ml/min, range 10; attenuation, ×4. Temperature programmed from 80 to 195°C at 4°C/min. The absence of a peak was taken to mean that the bisulfite reaction had been quantitative.

Regeneration of bisulfite adducts. Although our primary interest in conducting this study was for removal of carbonyl compounds from complex mixtures, an attempt was made in a number of instances to recover the carbonyl compounds from the adduct formed on the column. The procedure used was as follows: after the final washing of the bisulfite column, nitrogen was blown over the column to evapo-

rate residual solvent. Ammonia was passed over the column until the gas broke through (litmus) and the regenerated carbonyl was analyzed on the DNP and alumina columns.

Microgram column procedure. The procedure was scaled down so that it could be executed on a micro-bisulfite column contained in a melting point capillary. The end of a cut capillary is pushed into the impregnated Celite so that about 2 cm of the Celite is retained. Using the end of a paper clip or other suitable wire, the Celite is pushed into the capillary and then tamped with another tamping rod into a compact column approximately 1.5 cm long. One to 3 μl of the carbonyl-containing solution is injected onto the (previously unwetted) column followed by 2–3 μl of solvent from a clean syringe to rinse any residue adhering to the walls into the column. After 5 min about 10 μl of CCl_4 is injected onto the column to elute compounds not forming an adduct. Air or nitrogen pressure can be used to force the solution through the column. When about 8 μl (about 8 mm) of solvent have emerged, the solution is withdrawn as completely as possible with a hypodermic syringe for chromatographic analysis.

RESULTS AND DISCUSSION

Extraction of aldehydes. All normal chain saturated and unsaturated aliphatic and aromatic aldehydes (except piperonal) studied extracted quantitatively at the relatively rapid flow rate (7–12 min/0.5 ml), but aldehydes branched at the carbon atom alpha to the carbonyl group failed to extract quantitatively (Table I). In several instances no extraction occurred even using relatively slow flow rates (>19 min/0.5 ml).

Extraction of ketones. Of the aliphatic ketones tested only acetone reacted quantitatively at a relatively rapid flow rate (Table I). Only 10% of 2-butanone extracted at a comparable flow rate but extraction was quantitative when the flow rate was reduced to 27–28 min/0.5 ml. Methyl ketones with more than 4 carbon atoms did not extract at all, even at relatively slow flow rates.

The cyclic ketones, cyclopentanone, and cyclohexanone extracted readily on the column but cycloheptanone and cyclododecanone failed to extract even in traces.

The methyl esters of keto acids also were not extracted nor was 3-cholestanone. However, the two α,β-diketones studied (diacetyl and 2,3-heptanedione) extracted quantitatively.

Micro column extraction. The micro-columns in the melting point capillaries effectively removed the aldehydes tested. Figure 1 shows gas chromatographic patterns indicating the practically quantitative removal of cinnamaldehyde and palmitaldehyde from a mixture of

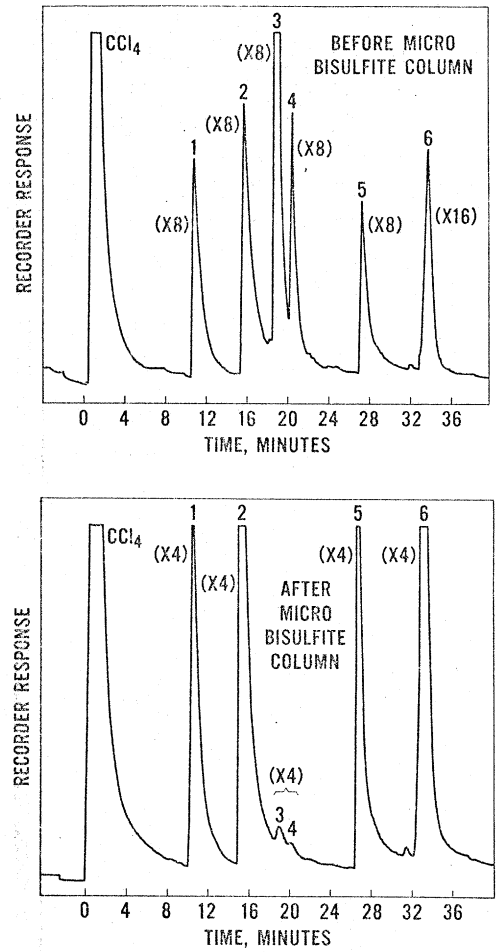

FIG. 1. Extraction of aldehydes from a mixture of classes by a microbisulfite column constructed in a melting point capillary. (1) 2-dodecanone, (2) 1-dodecanol, (3) cinnamaldehyde, (4) palmitaldehyde, (5) phenylacetic acid, (6) methyl-5,8,11,14,17-eicosapentaenoate.

carbonyl and other classes before and after contact with the bisulfite column.

Regeneration of carbonyl compounds. Recovery of carbonyls from the bisulfite column was variable on the compounds where attempts at regeneration were made. Carbonyls of relatively low volatility were recovered in good yield, while the more volatile carbonyls (acetone, 2-butanone) gave only fair yields. Furfural and 2,4-hexadienal were not recovered at all from the adduct, a phenomenon well known for α,β-unsaturated carbonyl compounds (*6*). Vanillin

and salicylaldehyde were also not recovered in readable amounts, probably due to their poor solubility in CCl_4.

Miscellaneous. The possibility that classes or members of a given class other than carbonyl compounds might be extracted by the bisulfite column was investigated. Aliphatic and aromatic alcohols and aliphatic esters passed the column quantitatively; fatty acids including acetic were not retained. Strong amines might be extracted, as the pH of a freshly made bisulfite column is 4.8–4.9, but these were not investigated.

SUMMARY

A procedure using a sodium bisulfite–Celite column was modified for extraction of micromole amounts of aldehydes and certain ketones from nonaqueous solution. Quantitative removal of saturated and unsaturated normal chain aliphatic and aromatic aldehydes takes place rapidly, but under the same conditions aldehydes branched on the alpha carbon atom fail to extract quantitatively or at all. Acetone, cyclopentanone and cyclohexanone were removed quantitatively but higher members in both classes were not. Vicinal diketones were extracted quantitatively. A scaled-down version conducted in a melting point capillary and suitable for microliter volumes containing microgram amounts of carbonyls also was tested.

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