

Methods for the Isolation and Characterization of Constituents of Natural Products

XVII. A Simple Microhydrogenation Technique

A considerable variety of hydrogenation techniques is available to the analyst (1-3, 4, 13). Relatively few of these, however, are applicable to microgram and submicrogram amounts of material. Of these, several hydrogenate in an instrument (3, 5, 6, 9, 11, 13) or with elaborate equipment (10), or are restricted to the use of paper (7) or thin-layer chromatography (8).

In a continuing effort to develop new and improved micro methods for isolating and characterizing constituents of natural products, a simple, rapid hydrogenation technique which utilizes a microcolumn of catalyst has been evolved. The method and the results are presented in this report.

MATERIALS AND APPARATUS

Celite 545 (Johns-Manville Co., Baltimore, MD); palladium chloride, 5% aqueous solution (Matheson, Coleman, and Bell, Norwood, OH); melting point capillaries, 100 mm long, 1.5-2.0 mm o.d. [Fisher Scientific Co., Silver Spring, MD (cat. no. 12-141)] cut approximately in half.

EXPERIMENTAL

The method is comprised of the following steps: (1) preparation of the catalyst; (2) construction of a microcolumn of the catalyst; (3) application of the sample to the column; (4) hydrogenation; (5) elution of the product(s) of hydrogenation; and (6) analysis of the effluent.

Preparation of the catalyst. One milliliter of the PdCl₂ solution is pipetted into a 4-in. mortar and ground with 2 g of Celite until the powder is homogeneously tan. It is then dried for 16 hr at 100°C, reground

briefly, and transferred to a 35 × 12-mm screw-capped specimen vial. The vial is stored in a desiccator containing "Drierite" when not in use. The catalyst is satisfactory to use for at least 14 weeks.

Construction of a microcolumn of catalyst. The end of a cut capillary is pushed into the catalyst until approximately 2.5–3.0 cm of powder are retained. Using the straight portion of an ordinary paper clip or other suitable tamping device, the powder is pushed up into the capillary so that approximately equal void space will be left above and below the column. With the tamping rod supporting the powder another tamper is used to press the powder into a compact column. Tamping should be done firmly so that the column will not separate or be blown out during hydrogenation, but not excessively tight so as to completely restrict the gas flow. A few practice runs will give sufficient experience to prepare satisfactory columns repeatedly.

Application of sample to the column. One–five microliters of a CCl_4 solution containing 0.5–40 μg of the unsaturated (or a mixture of saturated and unsaturated) compounds are injected onto the column from a hypodermic syringe. Any residue adhering to the walls is then washed into the column with two 2- μl aliquots of CCl_4 from a clean syringe. After these operations, there should remain some unwetted portion of the column.

Hydrogenation. The capillary is inserted into a capillary pipet holder such as that supplied with "Microcaps" (A. H. Thomas Co., Philadelphia, PA., cat. no. 7690-K10) or other suitable device and is attached to a source of hydrogen through a needle valve. With the needle valve slightly open the end of the capillary is immersed in a nonaqueous solvent to check for gas bubbles. As long as some gas is getting through the column, hydrogenation will occur within 5 min. The flow rate of hydrogen through the column will increase as the Pd^{2+} is reduced and regulation of the gas flow is usually not required during the hydrogenation period. The color of the entire column turns from tan to gray within 2 min as the Pd^{2+} is reduced and the gas is passed through the column for an additional 3 min after the color change.

Elution of hydrogenation products. The hydrogenated products are eluted from the column by injecting about 30 μl of CS_2 in 10- μl portions into the capillary. The first 4–5 μl (about 4 mm) of solvent emerging from the column contains all of the sample and is withdrawn with a hypodermic syringe. If desired, light air pressure may be used to force the solvent through the column.

Analysis of effluent. The effluent was examined by gas–liquid chromatography either on a 10 ft × 1/8 in stainless steel column containing 20% diethylene glycol succinate on 80–100 mesh Gas Chrom Q (for

long chain esters and alcohols) or on a 4 ft \times 1/8 in stainless steel column packed with 7.5% ethylene glycol adipate plus 2% H₃PO₄ on 90–100 mesh Anakrom ABS (for acids and shorter-chain compounds). A flame ionization detector was employed in both instances. Retention time was used as a means of identification. When no saturated analog was available, or when the analysis of polyunsaturated compounds was being undertaken, molecular weight determinations were carried out using a combination gas chromatograph–mass spectrometer (LKB-9000).

RESULTS AND DISCUSSION

The following unsaturated compounds were studied: methyl oleate, methyl elaidate, methyl palmitoleate, methyl *cis* vaccenate, methyl nervonate, methyl 10-undecenoate, methyl petroselinate, methyl linoleate, methyl linolenate, methyl arachidonate, methyl 4,7,10,13,16,19-docosahexaenoate, methyl 5,8,11,14,17-eicosapentaenoate, oleyl alcohol, elaidyl alcohol, vaccenyl alcohol, cinnamyl alcohol, citronellol, sorbic acid, petroselinic acid, 3-cyclohexen-1-carboxylic acid, and 1-cyclohexen-1-carboxylic acid. With the exception of the last compound, which hydrogenated to the extent of about 90%, all of the compounds were completely hydrogenated.

In addition, the methyl esters obtained from milk fat, a polyunsaturated milk fat, soybean, and safflower oils were investigated. These were all completely hydrogenated as judged by the disappearance of the oleate, linoleate, and linolenate peaks.

A mixture of methyl linoleate, linolenate, arachidonate, eicosapentaenoate, and docosahexaenoate (0.6 μ g each) and a mixture of methyl stearate (1.4 μ g), oleate (4.2 μ g), linoleate (5.6 μ g), linolenate (7.0 μ g), and arachidonate (9.4 μ g) were both completely hydrogenated. The GLC trace of the latter mixture before and after hydrogenation is reproduced in Fig. 1.

With the methyl esters obtained from oils and fats, we have found it preferable to use solutions containing not more than 6 μ g/ μ l and to inject 5 μ l onto the column rather than using more concentrated solutions and injecting a smaller volume.

Although we did not find it necessary to use longer and/or wider columns containing a larger charge of catalyst to accommodate more sample, there is no obvious reason why they should not be equally effective. Since the compounds are eluted from the column with the solvent front they can still be obtained in a small volume.

Compounds of relatively high volatility can be evaporated in the gas stream and may be partially or completely lost. This was the case with

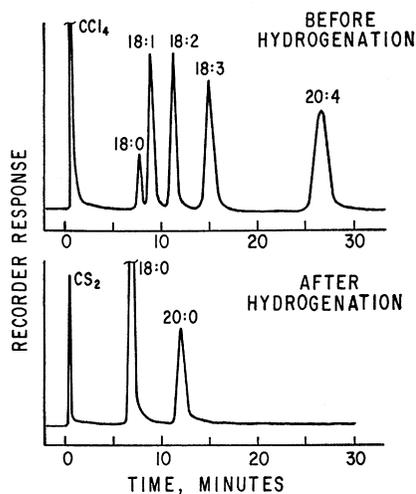


FIG. 1. Gas-liquid chromatograms of a mixture of methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, and methyl arachidonate before and after hydrogenation. Column, DEGS, 20% on Gas-Chrom Q. Attenuation, $\times 32$.

1-decene and 2-decene which were the only relatively volatile compounds which were investigated. Caproic acid, the hydrogenation product obtained from sorbic acid was recovered in good yield as judged from the gas chromatogram.

It should be pointed out that all of the monounsaturated compounds were completely reduced within 1 min after the reduction of Pd^{2+} . The polyunsaturated compounds, however, required a longer exposure, and the 3-min additional exposure to hydrogen following the reduction of the catalyst was routinely adopted.

SUMMARY

A simple, rapid procedure is described for complete hydrogenation of micro- and submicrogram amounts of unsaturated compounds. Hydrogenation is conducted on a microcolumn of Celite impregnated with palladium chloride and is complete within 5 min. The method has been applied to methyl esters of fatty acids, alcohols, and acids.

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