Identification of Unsaturated Dinitrophenylhydrazones by Partial Hydrogenation

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ABSTRACT

Small amounts of unsaturated carbonyl compounds, which originate from autoxidized fats, were converted into their corresponding 2,4-dinitrophenylhydrazones and subsequently partially hydrogenated. Neither hydrazine nor hydrogen gas and platinum oxide gave satisfactory results, but, using palladium on calcium carbonate as catalyst, the 2,4-dinitrophenylhydrazones could be hydrogenated partially. Only the double bonds of the aliphatic part of the molecule were reduced, whereas the carbon nitrogen double bond and the nitro groups were not attacked. After hydrogenation of 2,4-dinitrophenylhydrazones, the reaction mixture was analyzed by thin layer chromatography on a Kieselguhr plate impregnated with Carbowax. In this way, the chain length of the unsaturated carbonyl compound, as well as the number of double bonds, could be determined. Carbonyl conjugated double bonds appeared to be hydrogenated faster than isolated double bonds.

INTRODUCTION

Aliphatic unsaturated carbonyl compounds play an important role in the development of either desirable or undesirable flavors in oils, fats, and fat containing products. Often these carbonyls are characterized, after conversion into their 2,4-dinitrophenylhydrazones (DNPHs) and subsequent analysis on thin layer chromatography (TLC), by mass spectrometry and by IR spectroscopy. The position of the carbonyl conjugated double bonds can be located by measuring the maximum wavelength of absorption in UV light and, in case isolated double bonds are present, by the identification of the breakdown products which are formed after oxidation with osmium tetroxide (1) or ozone (2,3). However, before cleaving the DNPHs into fragments, it would be useful to know the chain length of the unsaturated aldehyde and the number of double bonds. This information could be obtained via partial hydrogenation of the DNPHs.

The partial hydrogenation of DNPHs should meet two requirements. First, neither the double bond between the N-atom and the C-atom nor the nitro groups should be hydrogenated. Second, the hydrogenation of the double bonds in the carbonyl part should not proceed too quickly but in such a way that the intermediate stages can be detected. These stages can be detected on Kieselguhr plates, impregnated with Carbowax 750 on which various classes of DNPHs, such as alkenals, alkadienals, and alkatrienals, of the same chain length can be separated (4).

EXPERIMENTAL PROCEDURES

Unsaturated Carbonyl Compounds

At our laboratory were synthesized: 5-*cis*-decenal (boiling point [bp] 57-60 C/1.5 mmHg; n_D^{20} 1.4431; 2,4-DNPH melting point [mp] 73.4-75 C); (2); 2-*trans*,6-*cis*-nonadienal (bp 97-99 C/15 mmHg; n_D^{20} 1.4738; 2,4-DNPH mp 112-113 C); (5); 2-*trans*,4-*cis*,7-*cis*-tridecatrienal (bp 90-92 C/0.8 mmHg; n_D^{20} 1.5100; 2,4-DNPH mp 94-95 C); synthesis analogous to that of 2-*trans*,4-*cis*,7-*cis*-decatrienal (6), 3-decen-2-one (bp 93.6-95.6 C/8 mmHg; n_D^{25} 1.4485; 2,4-DNPH mp 75-76 C).

Obtained from Fluka AG, Buchs, Switzerland: 3-buten-2-one (bp 79-80 C/760 mmHg; n_D^{20} 1.411; 2,4-DNPH mp 125-127 C).

Conversion into DNPHs

Small amounts of DNPHs were prepared by means of a DNPH reaction column (7).

TLC

The DNPHs dissolved in chloroform were spotted onto a Kieselguhr G chromatoplate impregnated with 33.3% Car-

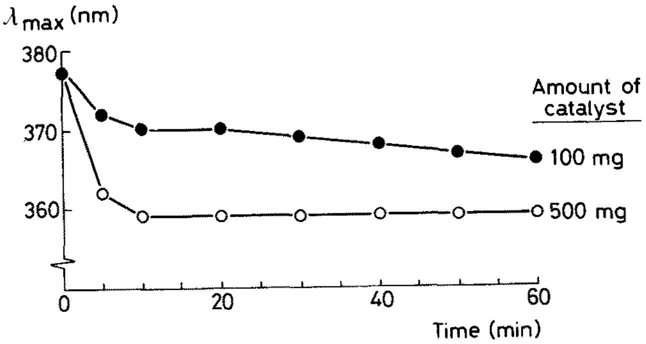

FIG. 1. Hydrogenation of 2,4-dinitrophenylhydrazones (DNPH) of 2-*trans*,6-*cis*-nonadienal with palladium (2 mg DNPH and catalyst in 25 ml chloroform).

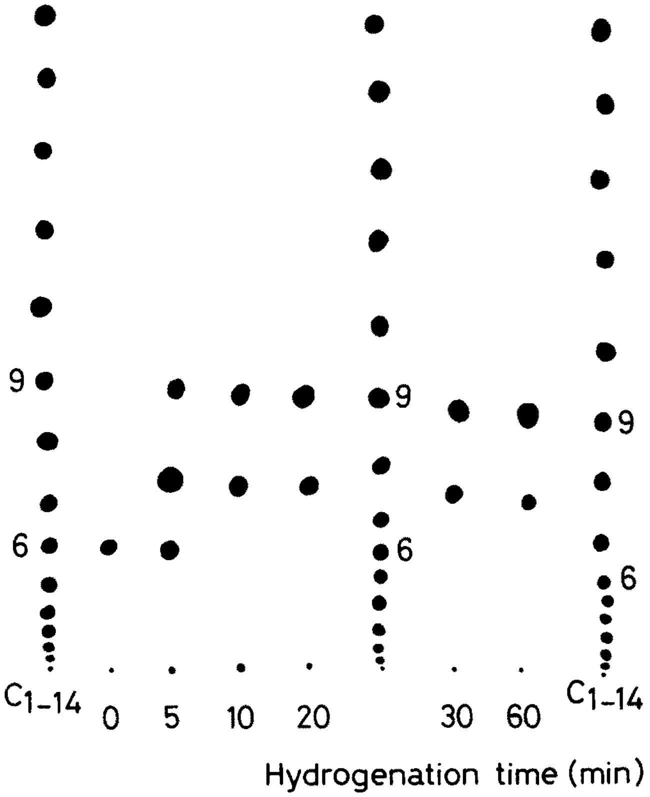

FIG. 2. Thin layer chromatogram of hydrogenated 2,4-dinitrophenylhydrazones (DNPH) of 2-*trans*,6-*cis*-nonadienal with 500 mg palladium-calcium carbonate on Kieselguhr-Carbowax 750.

bowax 750 (8). The reference compounds were applied to the plate from a benzene solution of DNPHs of saturated aldehydes with chain lengths from C1-C15 (Figs. 2,3) or of methyl ketones with chain lengths from C3-C11 (Fig. 4). Subsequently, ascending chromatography was applied using petroleum ether (bp 100-120 C) as eluant.

Partial Hydrogenation

The palladium catalyst was prepared as described by Lindlar (9), except that the poisoning with lead acetate was omitted. The palladium content is 2% calculated on calcium carbonate.

Ca. 10 μg DNPH was dissolved in 5 ml chloroform. Ca. 5 mg catalyst was added and 1 drop of ethanol. The air in the flask was displaced by leading a stream of hydrogen over the solution; after this replacement, the flask was closed and, while stirring, the DNPH was hydrogenated. The catalyst was filtered off, and, after concentration of the solution, the DNPH was spotted onto the chromatoplate.

RESULTS AND DISCUSSION

Preliminary experiments with hydrazine (10) showed that carbonyl conjugated double bonds could not be reduced. When hydrogen gas and Adams' catalyst (11) were used, the carbon nitrogen double bond also was hydrogenated. Palladium catalyst (9) and hydrogen gas did not attack this bond as followed from model experiments in which 2 mg 2,4-DNPH of decanal dissolved in 25 ml chloroform was hydrogenated with 100 mg catalyst for 60 min. When the extinction of the solution at 358 nm was measured every 10 min, no decrease was observed.

The influence of the amount of catalyst on the rate of hydrogenation of 2-*trans*,6-*cis*-nonadienal DNPH is given in Figure 1. With 100 mg palladium, after 60 min the maximum wavelength is still 366 nm, which means that, at least, a carbonyl conjugated DNPH is present. With 500 mg catalyst after 10 min the maximum wavelength of 358 nm has been reached from which can be concluded either that the saturated DNPH has been formed or that only the carbonyl conjugated double bond has been hydrogenated. Of course, these two phenomena also may occur simultaneously. When this expeirment, using 500 mg catalyst, was followed via a TLC analysis by spotting some drops of the reaction mixture onto a Kieselguhr plate impregnated with Carbowax 400, the results as shown in Figure 2 were obtained.

After 5 min hydrogenation, three spots became visible, but, after 10 min and longer, only two spots remained. Combined with the fact that the maximum wavelength after 10 min hydrogenation is 358 nm, it follows that these two spots are the DNPH of 6-*cis*-nonenal and of nonanal. This means that carbonyl conjugated double bonds are hydrogenated faster than isolated double bonds. This also is illustrated in Figure 3.

After 10 min the DNPH of 5-*cis*-decenal still is present, whereas after 5 min of hydrogenation there is only a faint spot left of the DNPH of 2-*trans*,4-*cis*,7-*cis*-tridecatrienal. So if there are indications about the presence of conjugated double bonds, a short time of hydrogenation, less than 5 min, is recommendable. The second spot obtained after hydrogenation of tridecatrienal comes from 2-*trans*,7-*cis*-C13, as shown by its UV absorption spectrum, the maximum wavelength being 366 nm. The third spot is 7-*cis*-C13, whereas the completely hydrogenated C13 aldehyde is found at the top of the plate.

The method is not limited to DNPHs of aldehydes, as is shown in Figure 4, where the TLC analysis is given of two ketone DNPHs after partial hydrogenation. In both cases, the double bond is located at the 3 position. It appeared that the terminal double bond is hydrogenated very fast as the original compound, 3-butene-2-one DNPH, could not be recovered after 5 min of hydrogenation.

From these experiments it may be concluded that the chain length of a straight unsaturated carbonyl compound and the number of double bonds can be determined after conversion into the corresponding 2,4-DNPH followed by partial hydrogenation with palladium catalyst and subsequent TLC analysis.

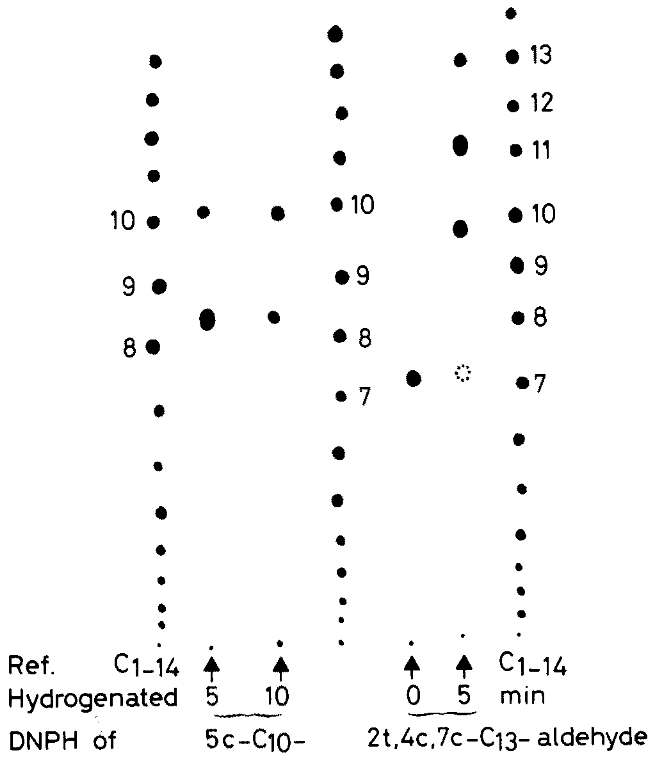

FIG. 3. Thin layer chromatogram of 2,4-dinitrophenylhydrazones (DNPHs) of 5-*cis*-decenal and 2-*trans*,4-*cis*,7-*cis*-tridecatrienal with 500 mg palladium on Kieselguhr-Carbowax 750.

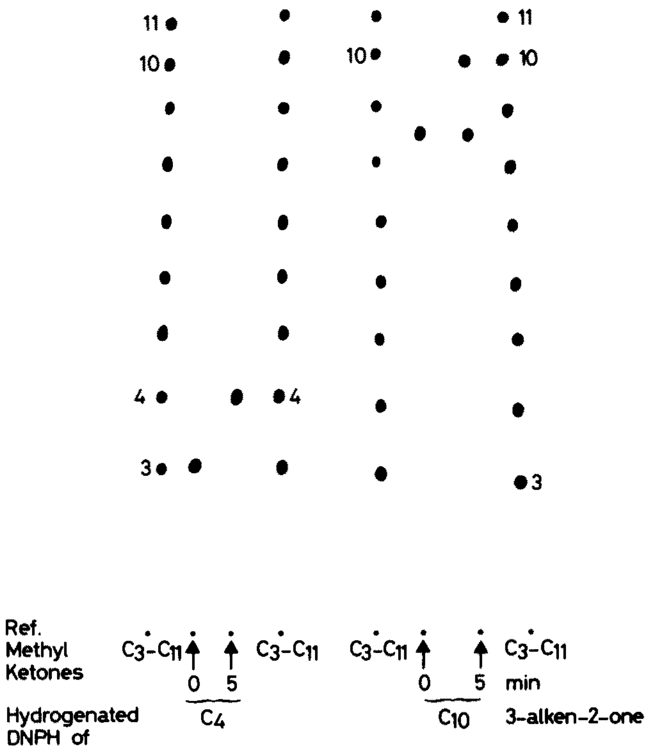

FIG. 4. Thin layer chromatogram of hydrogenated 2,4-dinitrophenylhydrazones (DNPHs) of 3-alken-2-ones with 5 mg palladium on Kieselguhr-Carbowax 750.

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