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Note

Reexamination of the acetylation of apiitol in the determination of apiose

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Apiitol, the sugar alcohol of apiose [3-*C*-(hydroxymethyl)-D-*glycero*-aldotetrose], is considerably more difficult to acetylate completely than sugar alcohols with only primary and secondary hydroxyl groups. We attempted to acetylate apiitol completely by modifying the conditions used by Blakeney et al. [1] for other sugars. When acetylation was performed at 35 °C for 1–20 h, a side-product, 1,2,4-tri-*O*-acetyl-3-*C*-(acetoxymethyl)-3-*O*-(methylthiomethyl)-D-*glycero*-tetritol [herein called 3-*O*-(methylthiomethyl)apiitol tetraacetate], was formed in substantial amounts [2]. Replacing dimethylsulfoxide (Me_2SO) with dimethylformamide avoided the formation of the side-product [2]. Harris et al. [3] reported that the methylthiomethyl ether side-product was not observed when the procedure of Blakeney et al. [1] was used with Me_2SO and the acetylation of apiitol was conducted at 40 and 80 °C for 90 min. In an attempt to reconcile the difference in results we used the conditions of Harris et al. [3] to acetylate apiitol. The methylthiomethyl ether side-product was again observed. At 40 and 80 °C the ether side-product was 30 and 38%, respectively, of the total peak area of the three compounds derived from apiose. The results at 40 °C were similar to those obtained previously at 35 °C [2]. Small amounts of the methylthiomethyl ether (0.2–1.8%) were detected even when acetylation was performed at 22 °C for 10 min. The amounts were dependent on how quickly the sample was cooled back to 22 °C after acetic anhydride was added. These results were obtained with the apiose used in the previous investigation [2] and with apiose purchased as the diisopropylidene derivative. Subjecting apiose to hydrolysis conditions [2 M trifluoroacetic acid (TFA), 120 °C, 1 h] before acetylation

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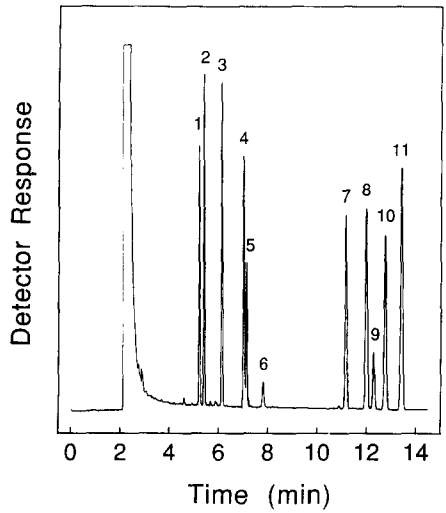

Fig. 1. Gas–liquid chromatogram of alditol peracetates and related compounds. The sample was prepared by the procedure of Blakeney et al. [1] except that the acetylation was performed at 40 °C for 90 min, and the extracted sample in dichloromethane was washed with 5 mL of water three times [11,12]. The sample was analyzed with a DB-225 capillary column operated isothermally at 230 °C. Peaks represent the peracetates of the following alditols, except as noted: 1, rhamnitol; 2, fucitol; 3, arabinitol; 4, xylitol; 5, apiitol; 6, apiitol tetraacetate; 7, mannitol; 8, galactitol; 9, 3-*O*-(methylthiomethyl)apiitol tetraacetate; 10, glucitol; 11, *myo*-inositol hexaacetate.

had no influence on the formation of the three products. The above results are consistent with our earlier results [2] but are contradictory to those of Harris et al. [3].

Harris et al. [3] stated that "the acetylation of sulphoxides in the Pummerer reaction is well documented, but side products are not formed when the reaction is conducted below 80°." Results in the literature do not support this as a general statement. The Pummerer reaction involving Me_2SO and acetic anhydride was shown to occur at 25 °C with formation of the expected sulfide [4]. The formation of the methylthiomethyl ether of a variety of alcohols, including sugars, from Me_2SO and acetic anhydride was also shown to occur at room temperature [5–9]. The rate of reaction was sufficient to detect the methylthiomethyl ether by GLC after 10 min. These results support our observation that the methylthiomethyl ether side-product was formed when more rigorous acetylation conditions were used in the procedure of Blakeney et al. [1].

Apiose can be determined quantitatively by measuring the peak area of apiitol pentaacetate after GLC [2]. Separation of apiitol and xylitol pentaacetates by GLC is difficult and a long sample separation time was required [2,3]. The method has been improved by reducing the separation time to less than 15 min while maintaining almost complete separation of apiitol and xylitol pentaacetates as well as complete separation of the other acetylation products (Fig. 1). For most samples, a DB-225 capillary column operated isothermally at 230 °C will give satisfactory results (Fig. 1). When a new DB-225 capillary column was used at 230 °C, separation of apiitol and xylitol pentaacetates was virtually complete (less than 1% of the total area of the two peaks

overlapped). The DB-225 column lost efficiency for separating the two pentaacetates very slowly. After hundreds of hours of use over 3.5 years, approximately 4% of the total area of the apiitol and xylitol pentaacetate peaks overlapped under the above chromatography conditions; however, there was substantial tailing of apiitol tetraacetate. The high column temperature was needed to separate the apiitol and xylitol pentaacetates. For samples where complete separation of apiitol and xylitol pentaacetates is necessary, both the DB-225 column operated at a lower temperature and a SP-2380 column gave baseline separation of these two pentaacetates as well as the other acetylation products stated in the legend of Fig. 1. The DB-225 column was operated isothermally at 170 °C for 55 min and then the temperature was increased at 1.5 °C/min to 220 °C and held. Under these conditions the order of elution of apiitol and xylitol pentaacetates was the reverse of that obtained when the column was operated isothermally at 230 °C (Fig. 1). The order of elution of the other products remained as shown in Fig. 1. The column chromatography time needed for a single sample was approximately 110 min. Baseline separation of the eight alditol peracetates plus *myo*-inositol hexaacetate was also achieved with a SP-2380 capillary column at 260 °C in 12 min. However, the SP-2380 column lost efficiency relatively rapidly (detectable after approximately 100 h of use) when operated at this temperature.

Harris et al. [3] proposed a method for quantitatively determining apiose based on the peak area of apiitol tetraacetate after GLC. Their method is more involved than the one based on apiitol pentaacetate and small amounts of the ether side-product may be formed.

1. Experimental

Sample preparation.—Apiose was isolated from parsley apiin [10]. 1,2:3,5-Di-*O*-isopropylidene-α-D-apiose was purchased from Pfanstiehl. Me_2SO, acetic anhydride, 1-methylimidazole, TFA and sodium borohydride from Sigma or Aldrich, and acetic acid and ammonium hydroxide were purchased new. Apiose (0.76 mg) and 1,2:3,5-di-*O*-isopropylidene-α-D-apiose (0.81 mg) were hydrolyzed with 2 M TFA at 120 °C for 1 h and the acid was removed with N_2 at 22 °C. Apiose (0.76 mg), hydrolyzed apiose, and hydrolyzed 1,2:3,5-di-*O*-isopropylidene-α-D-apiose were reduced separately and the alditols were acetylated by the method of Blakeney et al. [1], except that the acetylation was conducted at 22, 40, and 80 °C for 10 and 90 min. For samples acetylated at 22 °C, the vials were quickly cooled back to 22 °C by an air stream after acetic anhydride was added. A standard sample containing 0.11–0.12 mg of each of rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose, and *myo*-inositol and 0.46 mg of 1,2:3,5-di-*O*-isopropylidene-α-D-apiose was hydrolyzed with 2 M TFA at 120 °C for 1 h and the acid was removed as described above. The sugars and *myo*-inositol in the hydrolyzed standard were converted to products with the method of Blakeney et al. [1] except the acetylation was conducted at 40 °C for 90 min, and the extracted sample in dichloromethane was washed with 5 mL of water three times [11,12].

Gas chromatography.—Compounds were chromatographed on a DB-225 column [30 m × 0.25 mm (i.d.), 0.15 μm film thickness; J.&W.] attached to a Hewlett–Packard gas

chromatograph, Model 5840A, equipped with a splitter, a flame-ionization detector, and a 5840A data terminal. Helium was used as the carrier gas. When chromatography with the DB-225 column was performed isothermally at 230 °C, the sample size ranged from 1.2 to 4 μL, the carrier gas flow rate was 1.4 mL/min, and the split ratio was 1:5 (for apiose samples acetylated for 10 min at 22 °C) or 1:15 (other samples). For chromatography starting at 170 °C, the DB-225 column was kept at 170 °C for 55 min and then the temperature was increased at 1.5 °C/min to 220 °C and held. The sample size was 0.4 μL, the carrier gas flow rate was 1.7 mL/min, and the split ratio was 1:15. Compounds were chromatographed on a SP-2380 column [30 m × 0.25 mm (i.d.), 0.2 μm film thickness; Supelco] attached to the same system. Chromatography was performed isothermally at 260 °C. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. Apiitol tetraacetate, apiitol pentaacetate and 3-*O*-(methylthiomethyl)apiitol tetraacetate were identified by their retention times from GLC and by their mass spectra [2]. Mass spectrometry was performed as described previously [2].

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