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The acetylation of apiitol in the determination of apiose

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ABSTRACT

The complete acetylation of apiitol required 9 h when acetic anhydride at 120° was used and sodium acetate was the catalyst. Both apiitol pentaacetate and apiitol tetraacetate were detected before acetylation was complete. When the reaction was done in dimethyl sulfoxide, with 1-methylimidazole as the catalyst, a third compound was observed, and identified as 1,2,4-tri-*O*-acetyl-3-*C*-(acetoxymethyl)-3-*O*-(methylthiomethyl)-D-*glycero*-tetritol [3-*O*-(methylthiomethyl)apiitol tetraacetate] by gas-liquid chromatography and mass spectrometry. In *N*,*N*-dimethylformamide, with 1-methylimidazole as catalyst, the acetylation of apiitol was essentially complete in 4 h at 85°, and the formation of methylthiomethyl ether was avoided. A method for preparing alditol acetates using 1-methylimidazole as the catalyst, and suitable for samples containing apiose as well as ordinary sugars, is described. The separation of apiitol pentaacetate from xylitol pentaacetate by gas-liquid chromatography proved difficult. However, a virtually complete separation of the peracetates of apiitol and xylitol as well as complete separation of those of rhamnitol, fucitol, arabinitol, mannitol, galactitol, glucitol, and *myo*-inositol, plus apiitol tetraacetate and 3-*O*-(methylthiomethyl)apiitol tetraacetate, was accomplished with a 30 m × 0.53 mm (i.d.) SP-2380 column in 49 min, and on a 30 m × 0.75 mm (i.d.) SP-2330 column in 82 min. A complete separation of apiitol and xylitol pentaacetates as well as four other alditol peracetates was obtained with a 60 m DB-1 column in 15.2 min, however this column did not resolve the acetates of fucitol and arabinitol. A variety of other columns and column conditions were ineffective.

INTRODUCTION

Apiose [3-*C*-(hydroxymethyl)-D-*glycero*-aldotetrose] is widely distributed in the plant kingdom[1]. It is present both in cell-wall polysaccharides and in non-cell-wall compounds[2]. It is therefore of interest when the sugar composition of plant cell walls and cell-wall fractions is determined, typically by the preparation of alditol acetates after acid hydrolysis of the sample. All of the known sugars present in plant cell-wall material, except apiose and aceric acid[3], have only primary and secondary hydroxyl groups, and consequently their alditols are acetylated relatively easily. Apiose and aceric acid, on the other hand, have tertiary hydroxyl groups, which past experience has shown are difficult to acetylate when the more common catalysts such as pyridine are used[4]. The conditions for complete acetylation of apiitol have not been established, although the assumption may have been made that the published conditions for the acetylation of other alditols also gave complete acetylation of apiitol. We investigated the acetylation of apiitol by two methods currently used to acetylate alditols[5-7], and found that complete acetylation in both cases required considerably more rigorous

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conditions than those needed for sugar alcohols having only primary and secondary hydroxyl groups. We also found that when the procedure of Blakeney *et al.*[7] was used under conditions that gave complete conversion of apiitol, substantial amounts of 1,2,4-tri-*O*-acetyl-3-*C*-(acetoxymethyl)-3-*O*-(methylthiomethyl)-D-*glycero*-tetritol [herein called 3-*O*-(methylthiomethyl)apiitol tetraacetate] were formed as a side-product.

EXPERIMENTAL

Materials. — Apiose was isolated as described previously[8]. Other sugars and *myo*-inositol were obtained from Pfanstiehl Laboratories, Inc. 1-Methylimidazole, 4-dimethylaminopyridine, dimethyl sulfoxide, and fully deuterated acetic anhydride were obtained from Aldrich Chemical Co., Inc. *N*,*N*-Dimethylformamide was purchased from Sigma Chemical Co. *myo*-Inositol hexaacetate (m.p. 213–214° was prepared by a modification of the procedure described by Wachowiak and Conners[6] for glucose pentaacetate. Columns packed with SP-2330, SP-2380, and DB-1 were purchased from Supelco, Inc. and J & W Scientific, Inc., respectively.

Preparation of alditol acetates. — Alditol acetates were prepared by the following four procedures: (1) Albersheim *et al.*[5], (2) Blakeney *et al.*[6,7], (3) Steglich and Höfle[9], and (4) a modification of the procedure of Blakeney *et al.*[6,7] The particular procedure used, along with any changes, is stated when the results of individual experiments are described. In the preparation of individual samples, 0.89–1.78 μmol of apiose and 0.82–1.0 μmol of rhamnose, fucose, arabinose, xylose, galactose, glucose, and mannose were used*. Except where indicated otherwise, *myo*-inositol was added to the samples as an internal standard.

The procedure of Blakeney *et al.*[7] was modified as follows so that complete acetylation of apiitol was achieved without formation of any side-product (acetylation procedure IV). The sample of apiose was in 0.2 mL of M ammonium hydroxide, and after 1 mL of 2% (w/v) $NaBH_4$, in DMF was added it was kept for 60 min at 40°, cooled to 22°, acidified with 0.2 mL of glacial acetic acid, and mixed. For acetylation, 0.2 mL of 1-methylimidazole and 2 mL of acetic anhydride were added and the sample was mixed and heated for 4 h at 85°. Five mL of water was added to the solution at 22° and after mixing and cooling to 22°, 1 mL of dichloromethane was added. The sample was mixed, then centrifuged, and the organic phase used for gas chromatography. The modified procedure was also tested with rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose, and *myo*-inositol.

Gas chromatography. — Gas-liquid chromatography of alditol acetates was performed initially with a Varian Aerograph, Series 2100, and more recently with a Varian Model 3700 instrument, each equipped with a flame-ionization detector. A

*Abbreviations: e.i., electron impact; c.i., chemical ionization; M^+, molecular ion; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; all sugars have the D configuration except for L-rhamnose, L-fucose, and L-arabinose.

capillary glass column (30 m × 0.75 mm, i.d.; film thickness 0.2 μm), coated with SP-2330 and a fused-silica column (30 m × 0.53 mm, i.d.; film thickness 0.2 μm), coated with SP-2380, were used. Samples were injected directly into the SP-2330 column. When the SP-2380 column was used the Model 3700 chromatograph was equipped with a splitter, with the split ratio ranging from 1:7 to 1:15. With the SP-2330 column, nitrogen was used as both the carrier and makeup gas, usually at flow rates of 5 and 40 mL. min^{-1}, respectively, while with the SP-2380 column helium was the carrier gas and the flow rate normally was 5 $mL.min^{-1}$. Data from these two columns were collected with a Hewlett–Packard Reporting Integrator, Model 3390A. Alditol acetates were also separated on a DB-1 fused-silica capillary column (60 m × 0.25 mm, i.d.; film thickness 0.1 μm) attached to a Hewlett-Packard gas chromatograph, Model 5840A, equipped with a splitter, a flame-ionization detector, and a 5840A data terminal. Helium was the carrier gas and the flow rate was 0.8 $mL.min^{-1}$. The split ratio usually was 1:14.

Samples injected into gas chromatographs ranged in size from 0.1 to 1.0 μL when no splitter was used and from 1 to 2.5 μL with a splitter.

Mass spectrometry. — E.i. and c.i. mass spectrometry were performed with a JEOL HX110-HF, double-focusing mass spectrometer interfaced to a Hewlett–Packard gas chromatograph, Model 5890A, which was equipped with a splitless injector and a DB-1 fused silica column (15 m × 0.53 mm, i.d.; film thickness 1.5 μm). Helium was the carrier and makeup gas; the flow rates were 10 and 20 $mL.min^{-1}$, respectively. The ion source temperature was 200° and the ionizing voltage was 70 eV. For c.i. mass spectrometry, ammonia was the reagent gas.

RESULTS

Acetylation with sodium acetate as catalyst. — Complete acetylation of apiitol required 9 h when acetic anhydride at 120° was used and sodium acetate was the catalyst[5]. Samples prepared from apiose by the procedure of Albersheim *et al.*[5] were analyzed with the 30 m SP-2330 column at 200° (see Experimental) at acetylation times of 20 min, 40 min, 1, 1.5, 2, 3, 4, 5, 7, and 9 h. The observed values for apiitol pentaacetate and apiitol tetraacetate were 10.7, 41.4, 59.5, 69.3, 72.3, 85.7, 98.0. 97.0, 99.2, and 100% and 89.3, 58.6, 40.5, 30.7, 27.7, 14.3, 2.0, 2.9, 0.8, and 0%, respectively, calculated from the area under each chromatographic peak, with the sum of each pair of areas set equal to 100%. The acetates were identified by gas–liquid chromatography and mass spectrometry (Figs. 1a and 1b). Based on the fragmentations proposed, ions at *m/z* 289, 303, 319, 347, and 362 would be unique to the pentaacetate. Of these only *m/z* 289 was present in substantial amounts; it had a relative intensity of 16.8 with respect to the base peak* at *m/z* 187. The other ions were not present except for *m/z* 303, having intensity 0.82. In the spectrum of the tetraacetate derivative all these ions were un-

*In this paper: (1) the base ion (peak) was assigned a value of 100, (2) relative intensities are with respect to the intensity of the base ion, and (3) ions with *m/z* of 50 or less were not considered.

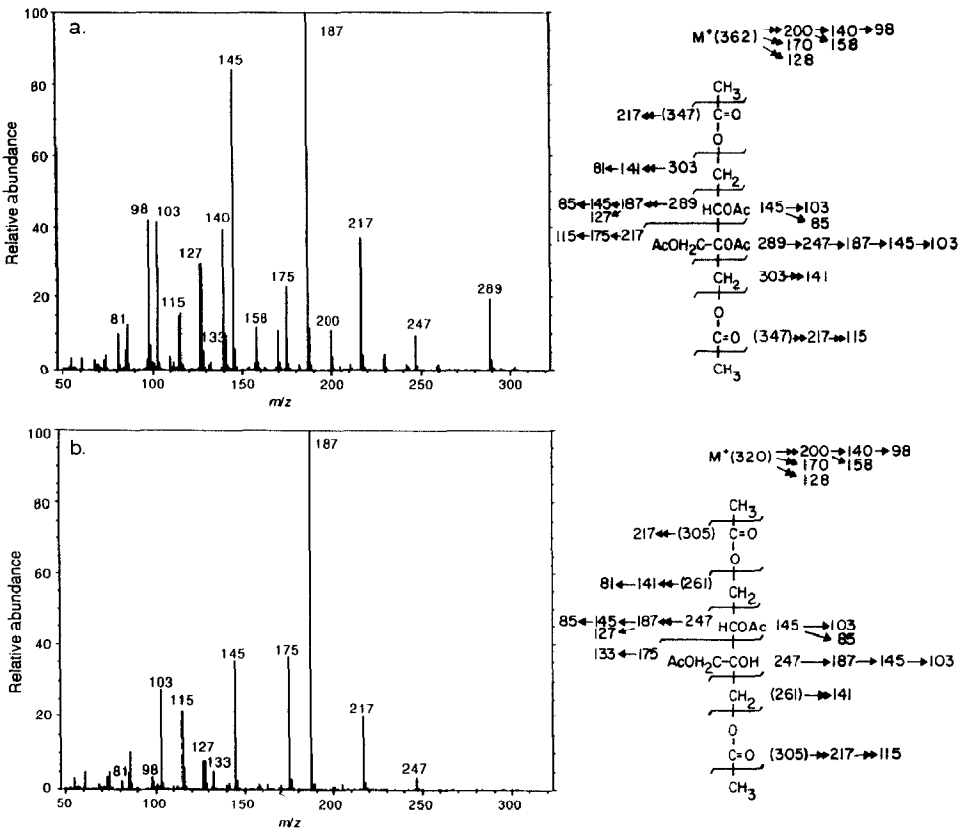

Fig. 1. E.i. mass spectra of (a) apiitol pentaacetate, and (b) apiitol tetraacetate. See Experimental for procedure. Parentheses mean the ion was not detected. Double-headed arrows represent two or more steps. Sequences to minor ions are not shown.

detectable or scarcely apparent. Ion 289 therefore can be used to distinguish between apiitol pentaacetate and apiitol tetraacetate.

According to the fragmentations proposed, the ions at m/z 133, 159, 218, 261, 277, 305, and 320 are unique to the tetraacetate. Of these only 133 and 218 were present in the spectrum of the tetraacetate at intensities greater than 0.85; their peak heights were 5.3 and 2.3, respectively. In the spectrum of the pentaacetate, the ions at m/z 133 and 218 had relative intensities of 2.8 and 3.8; the remaining ions were of intensity 0.38 or less except for m/z 159 at 2.2. The relative intensity of ion 133 was always 2–3 times greater in the spectra of the tetraacetate than in the spectra of the pentaacetate. The reactions proposed to account for the spectra of the two derivatives accounted for 92 and 91%, respectively, of the total ions detected (background ions included, $\leq 0.05\%$ of the total ion current).

Acetylation with 1-methylimidazole as catalyst — When apiitol and acetic anhydride were heated at 35° in the presence of 1-methylimidazole and DMSO for various

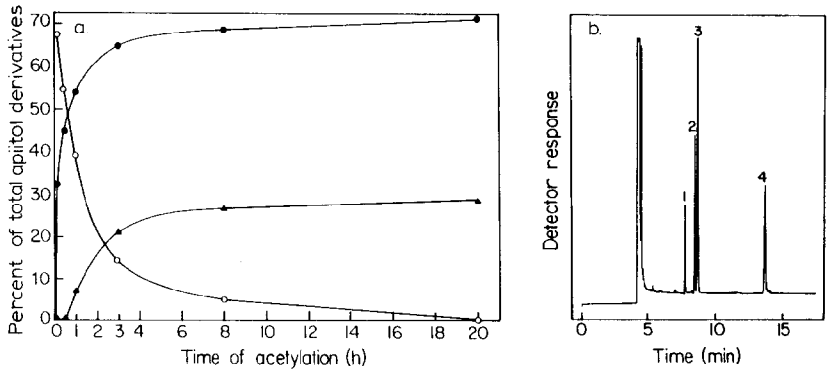

Fig. 2. Acetylation of apiose by the procedure of Blakeney *et al.*[7]. Samples were prepared from apiose as described[7], except that acetylation was performed at 35° for the times indicated. A single extraction with dichloromethane was performed, resulting in underestimation of the proportions of apiitol tetraacetate (see Results). Samples were analyzed with the 60 m DB-1 column as described in the Experimental part. Column operation was isothermal at 200°. (a) Time course of formation of products, showing apiitol tetraacetate (o), apiitol pentaacetate (•), and 3-*O*-(methylthiomethyl)apiitol tetraacetate (▲) as percentages of the total dichloromethane-extracted material at each time point. (b) Gas-liquid chromatogram of the products. Acetylation time for this sample was 5 h, and xylitol pentaacetate in dichloromethane was added to the mixture before g.l.c. Compounds 1, 2, 3, and 4 are apiitol tetraacetate, xylitol pentaacetate, apiitol pentaacetate, and 3-*O*-(methylthiomethyl)apiitol tetraacetate, respectively.

time periods the results shown in Fig. 2a were obtained. A typical separation of the three compounds detected is shown in Fig. 2b. Two of the compounds were identified as apiitol pentaacetate and apiitol tetraacetate by their e.i. mass spectra. These were as shown in Figs. 1a and 1b, with the ions at *m/z* 289 and 133 having the same relative intensities in the respective spectra. The c.i. mass spectrum of the compound considered to be apiitol pentaacetate showed ions at *m/z* 380 ($M + NH_4^+$), 363 ($M + H^+$), and 303; their relative intensities were 100, 0.31, and 55, respectively. When fully deuterated acetic anhydride was used, ions at *m/z* 395 ($M + NH_4^+$), 378 ($M + H^+$), and 315 had relative intensities 28.5, 0.2, and 100, respectively. The c.i. mass spectrum of the compound considered to be apiitol tetraacetate showed ions at *m/z* 338 ($M + NH_4^+$), 321 ($M + H^+$), and 303 at relative intensities 62, 2.7, and 100, respectively. Fully deuterated acetic anhydride gave ions at *m/z* 350 ($M + NH_4^+$), 333 ($M + H^+$), and 315 with intensities 7.5, 0.84, and 100. These data confirm the identity of two of the products (Fig. 2a) as apiitol pentaacetate and apiitol tetraacetate, as well as provide confirmation of the identity of the two compounds formed from apiose when the procedure of Albersheim *et al.*[5] was used.

The third compound in Fig. 2a was identified as 3-*O*-(methylthiomethyl)apiitol tetraacetate by mass spectrometry. The base ion in the e.i. spectrum (Fig. 3) had *m/z* of 61 and probably was mainly $CH_3SCH_2^+$. The fragmentations proposed accounted for 87.5% of the total ions in the spectrum (background included). The c.i. mass spectrum of the compound showed ions at *m/z* 398 ($M + NH_4^+$), 381 ($M + H^+$), and 303 at relative intensities of 100, 0.38, and 55, respectively. Fully deuterated acetic anhydride

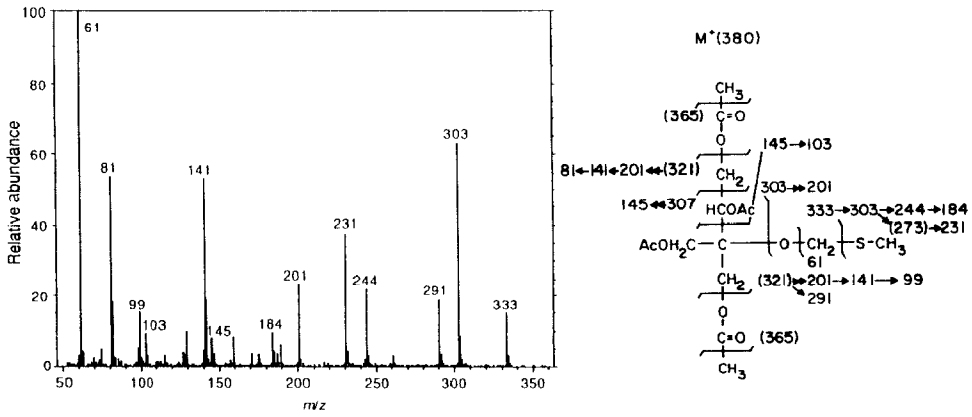

Fig. 3. E.i. mass spectrum of 3-*O*-(methylthiomethyl)apiitol tetraacetate. See legend to Fig. 1.

gave ions at m/z 410 ($M + NH_4^+$), 393 ($M + H^+$), and 315, having intensities 24.8, 0.47, and 76.2, respectively. Ion 61 was now the base ion. In addition, the following data further support the identification of the third compound: (1) the compound was not formed when apiitol was acetylated by procedure IV (information presented later), (2) the data in Fig. 2a show that formation of the compound ceased when apiitol tetraacetate was no longer present, (3) the compound was not formed when the other tested acetylation procedures were used with apiitol, and (4) it was not formed when alditol peracetates of rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose were prepared by the procedure of Blakeney *et al.*[7], either as described in the reference or by acetylation for 4 h at 85°.

Acetylation with 4-dimethylaminopyridine as catalyst. — When apiitol was acetylated by acetic anhydride with 4-dimethylaminopyridine as the catalyst[9], only apiitol pentaacetate and apiitol tetraacetate were observed (data not presented). Under our best conditions (9.5 h, 22°, no pyridine added), 96% of the material detected by the gas chromatograph was apiitol pentaacetate, and 4% was apiitol tetraacetate. The yield of apiitol pentaacetate was not increased by acetylating at 40°, increasing the acetylation time to 24 h, adding pyridine together with 4-dimethylaminopyridine, or various combinations of these.

Acetylation procedure for apiose and other sugars — The modification of the procedure of Blakeney *et al.*[7] permitting its use with apiose consisted of replacing DMSO with DMF and increasing the time and temperature of acetylation to 4 h and 85°, respectively. With the new procedure (acetylation procedure IV), greater than 99% of the apiitol recovered was fully acetylated and no side-products were observed. The time course is given in Fig. 4. A small amount of apiitol tetraacetate was still present after 4 h of acetylation at 85° (0.56%) and some was present even after 18 h at 85° (0.17%). However, since these amounts are less than 1%, acetylation for 4 h at 85° should be acceptable for virtually all work.

The degree of reduction of sugars treated with $NaBH_4$ in DMF was examined

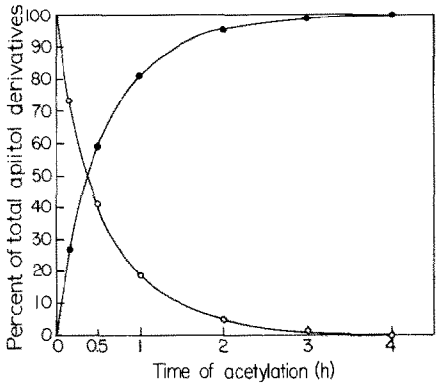

Fig. 4. Formation of apiitol pentaacetate from apiitol and acetic anhydride with acetylation procedure IV. Samples were prepared as described in the Experimental part, except that acetylation times were varied. A single extraction with dichloromethane was performed, resulting in underestimation of the apiitol tetraacetate. Samples were analyzed with the SP-2380 column operating isothermally at 200°. The curves show apiitol tetraacetate (o) and apiitol pentaacetate (●).

after 15, 30, 60, and 90 min at 40°. A sample was prepared that contained the seven sugars listed in the Experimental but no apiose; a second contained only apiose. Sugars were reduced and subjected to acetylation procedure IV, except that the reduction time was varied as indicated above and the sample containing the seven sugars was acetylated for 10 min at 22°. *myo*-Inositol hexaacetate (0.15 mg) was added with the dichloromethane. The relative peak areas of the peracetates of apiitol, xylitol, mannitol, galactitol, and glucitol with respect to *myo*-inositol hexaacetate were maximal at 15 min of reduction and did not change more than ±4% between 15 and 90 min. The relative peak areas of the peracetates of rhamnitol, fucitol, and arabinitol at 15 min were 93, 94, and 93%, respectively, of those obtained at 90 min. The relative peak areas of the peracetates of rhamnitol, fucitol, arabinitol, and the other alditols were not different by more than ±4% at 60 and 90 min. These results showed reduction in DMF proceeded more rapidly than in DMSO[7].

When the acetylation mixture of Blakeney *et al.*[7] was held at 22° for extended periods or heated at 85°, the solution darkened[10] owing to a reaction between 1-methylimidazole and acetic anhydride. When 0.2 mL of 1-methylimidazole, 2 mL of acetic anhydride, and 1 mL of DMF were heated for 4 h at 85° with 0.1, 0.15, or 0.2 mL of M NH_4OH neutralized with glacial acetic acid, the absorbance of the solutions at 420 nm was 1.85, 0.47, and 0.29, respectively. Color formation also decreased when decreasing amounts of 1-methylimidazole were used. Addition of mannitol (a representative alditol) and *myo*-inositol together to the acetylating solution or substituting water for the neutralized ammonium hydroxide solution had no significant effect on color formation.

Completeness of extraction by dichloromethane was examined. Apiose was treated by acetylation procedure IV, except that the sample was heated only 15 min at

85°. A mixture of rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose was subjected to acetylation procedure IV as described. Samples were prepared in duplicate. *myo*-Inositol hexaacetate (0.15 mg) was added with the first 1.0 mL of dichloromethane. Each sample was extracted successively four times, each time with 1.0 mL of dichloromethane, and an aliquot of each organic phase was analyzed by gas chromatography. The recoveries, based on total amount extracted, of compounds present in organic phases 1 through 4, respectively, were: (1) apiitol tetraacetate, 38, 40, 16, and 5.8%, (2) apiitol pentaacetate, 81, 19, 0, and 0%, and (3) *myo*-inositol hexaacetate, 86, 13, 1.0, and 0.2%. The recoveries of the peracetates of rhamnitol, fucitol, arabinitol, xylitol, mannitol, galactitol, and glucitol in organic phases 1 through 3 ranged from 78 to 87, 13 to 21, and 0 to 3%, respectively, and were zero in organic phase 4. Clearly, if apiose is incompletely acetylated and a single extraction with dichloromethane is performed, the amount of apiose originally present in the sample will be seriously underestimated.

When acetylation procedure IV was used with rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose, the yields of alditol acetates ranged from 104 to 119% of those obtained with the original procedure[7]. When acetylation procedure IV was used, but with a reaction time of 10 min at 22°, the yields of the individual alditol acetates were basically the same as from the original procedure[7].

Separation of alditol acetates. — Complete separation of apiitol and xylitol pentaacetates was achieved on a 60 m DB-1 capillary column (Fig. 2b). When the DB-1 column was used with a temperature program designed for rapid elution, apiitol pentaacetate, apiitol tetraacetate, *myo*-inositol hexaacetate, and the alditol peracetates of rhamnose, xylose, mannose, glucose, and galactose were separated in 15.15 min. The acetates of fucitol and arabinitol were separated from the others but not from each other, and mannitol hexaacetate was not separated from 3-*O*-(methylthiomethyl)apiitol

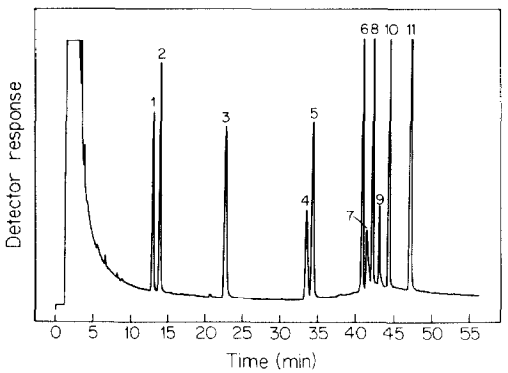

Fig. 5. Gas-liquid chromatogram showing the separation of alditol peracetates and related compounds. The sample was prepared by the procedure of Blakeney *et al.*[7], except that acetylation was for 15 min at 85°. The sample was analyzed with the SP-2380 column programmed to hold at 170° for 32 min, then increase 4° min^{-1} to 210°, and hold at 210°. Peaks represent the peracetates of the listed alditols, and other compounds as noted: 1, rhamnitol; 2, fucitol; 3, arabinitol; 4, apiitol; 5, xylitol; 6, mannitol; 7, apiitol tetraacetate; 8, galactitol; 9, 3-*O*-(methylthiomethyl)apiitol tetraacetate; 10, glucitol; 11, *myo*-inositol.

tetraacetate. Virtually complete separation of apiitol and xylitol pentaacetates as well as separation of the alditol peracetates of the other six sugars listed above, apiitol tetraacetate, 3-*O*-(methylthiomethyl)apiitol tetraacetate, and *myo*-inositol hexaacetate was achieved in 49 min with the SP-2380 column (Fig. 5). A similar separation of these compounds was achieved in 80 min with the SP-2330 column. The order of elution of the compounds from the latter column was the same as that shown in Fig. 5, except that apiitol tetraacetate eluted immediately before mannitol hexaacetate rather than after. The use of a variety of other carrier-gas flow rates and temperatures with these two columns did not improve the separation of apiitol and xylitol pentaacetates. A number of other columns, both packed and capillary, were tested but gave either no or only partial separation of apiitol and xylitol pentaacetates.

DISCUSSION

Complete acetylation of apiitol is considerably more difficult to achieve than complete acetylation of alditols having only primary and secondary hydroxyl groups. This is shown by the requirement for a 9-hour reaction period at 120° when the procedure of Albersheim *et al.*[5] is applied to apiitol.

The procedure described by Blakeney *et al.*[7] is not suitable for the acetylation of either apiitol or presumably any other alditol possessing a tertiary hydroxyl group because of formation of a methylthiomethyl-ether derivative as a side-product. This might be expected, because the acetylation mixture is analogous to reaction mixtures containing DSMO and acetic anhydride that are used in organic synthesis to oxidize primary and secondary alcohols to the corresponding carbonyl compounds[11,12]. A common side-product in these oxidations is the methylthiomethyl ether of the alcohol[11,12]. In the usual applications of the Blakeney *et al.* procedure acetylation evidently proceeds much more rapidly than oxidation, consequently oxidized sugars have not been observed. When tertiary alcohols are subjected to these conditions oxidation cannot occur, allowing methylthiomethyl ether formation to proceed, often in high yield[13,14]. Moreover, the formation of the methylthiomethyl ether is facilitated by acetic acid[13], which is present in substantial amounts in the Blakeney *et al.*[7] acetylation mixture. We found that heating the acetylation mixture for 4 h at 85° resulted in an organic phase 3-fold larger in volume than those obtained from samples kept at 22° for 10 min or 4 h, perhaps because of the formation of acetoxymethyl methyl sulfide by Pummerer rearrangement[15] of acetoxydimethylsulfonium acetate derived from the DMSO and acetic anhydride present.

Color formation in the acetylation solution can be diminished by increasing the volume of the aqueous sample solution or by decreasing the amount of 1-methylimidazole used, or both. However, both changes have potential for decreasing the yield of alditol acetates. The quantities specified in acetylation procedure IV provide for a full yield of alditol acetates while minimizing color formation. Moreover, this procedure should be suitable for alditols possessing tertiary hydroxyl groups.

The mass spectra presented in Figs. 1a, 1b, and 3 can be explained in large part by

reactions reported in the literature. Particular relevant are processes resulting in the loss of acetic acid, ketene, formaldehyde, acetic anhydride, and water[16,17], but other reactions are also involved. In this connection it is of interest to consider the ion of *m/z* 244, found in the spectrum of 3-*O*-(methylthiomethyl)apiitol tetraacetate, which we suggest must be formed from *m/z* 303. The change involves conversion of a cation having an even number of electrons (*m/z* 303) into an odd-electron cation-radical (*m/z* 244) and a radical (*m* 59), a process that is usually considered energetically unfavorable. However it is not always unfavorable; a recent survey revealed a large number of examples of this type of conversion[18]. The e.i. mass spectrum of apiitol pentaacetate is similar to those of the common alditol acetates, in particular xylitol and arabinitol pentaacetates. The interpretation of these spectra has been extensively discussed[16,17,19].

Separation of apiitol pentaacetate from xylitol pentaacetate by gas chromatography is difficult, but complete or almost complete resolution of the two was achieved with three columns. On the polar columns, SP-2330 and SP-2380, apiitol tetraacetate overlapped other compounds because of tailing (Fig. 5). However, when acetylation procedure IV is used apiitol tetraacetate will not be present.

In previous analyses of natural materials apiose may not have been detected either because the acetylation of apiitol was incomplete and the resulting apiitol tetraacetate was not detected (or not identified), or because apiitol pentaacetate was not separated from xylitol pentaacetate, or both. With the methods described here, the separation, identification, and quantitative determination of apiose is possible.

ACKNOWLEDGMENTS

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