Phytochemistry, 1979, Vol. 18, pp. 1393–1394. Pergamon Press Ltd. Printed in England.

SUGAR-RELATED HYDROXY ACIDS FROM *PHASEOLUS* AND *TRIFOLIUM* SPECIES

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(*Received* 3 *January* 1979)

Key Word Index—*Phaseolus vulgaris*; *Trifolium incarnatum*; Leguminosae; bean; clover; hydroxy acids; aldonic acids.

Abstract—Hydroxy acids isolated from leaves of French bean (*Phaseolus vulgaris*) and clover (*Trifolium incarnatum*) were analysed by GLC as trimethylsilyl derivatives and identified by MS. Large amounts of a 2-*C*-methyltetronic acid and appreciable amounts of gluconic acid and of a 2-*C*-(hydroxymethyl)pentonic acid were found from French bean. Glyceric acid was the predominant acid from clover but the presence of several other acids, e.g. threonic and malic acids, was also demonstrated.

INTRODUCTION

Hydroxy acids related to sugars (hexonic and pentonic acids and related compounds) were first isolated from leguminous plants by Soldatienkov and Mazurova [1] who named them 'acids of primary oxidation of sugars'. They may constitute almost half of the total acidity of the leaves and several percent of the dry mass of these plants. In a previous study, the acids were isolated from various leguminous plants and analysed by TLC [2]. This method, like those used by the Soldatienkov group, did not permit final identifications of several important acids. The present investigation by GC–MS was therefore initiated primarily with the purpose to determine the individual structures of the unknown major components.

RESULTS

The acids isolated from bean and clover as precipitate (P) and filtrate (F) fractions in 80% aqueous ethanol gave rise to the chromatograms reproduced in Fig. 1 on temperature-programmed GC analysis after conversion to trimethylsilyl derivatives [3]. The samples were also studied by GC–MS and the compounds listed in Fig. 1 were identified. The identifications were made by comparison with reference retention data [4] and reference mass spectra [5]. The chromatographic properties of the 2-*C*-methyltetronic acid indicate that the acid is 2-*C*-methylerythronic acid. The 2-*C*-(hydroxymethyl)-pentonic acid might be expected to be configurationally related to the major sugar metabolites and therefore to have the *ribo* or *arabino* configuration. A reference spectrum of a 2-*C*-(hydroxymethyl)pentonic acid has been published [6]. The structures of the unusual branched acids are shown in Fig. 2.

From the upper part of Fig. 1 it is seen that the 2-*C*-methyltetronic acid is by far the most prominent acid obtained from French bean. Gluconic acid and the 2-*C*-(hydroxymethyl)pentonic acid are the second and third most prominent acids, but the amount of these acids in 'bean F' is very small considering the eight times decreased attenuation relative to the 2-*C*-methyltetronic acid. The compound in 'bean P', eluted after the acids, was identified as an inositol (probably *myo*-inositol) by MS and does not belong to the acid compounds. Studies by TLC demonstrate that other varieties of French bean

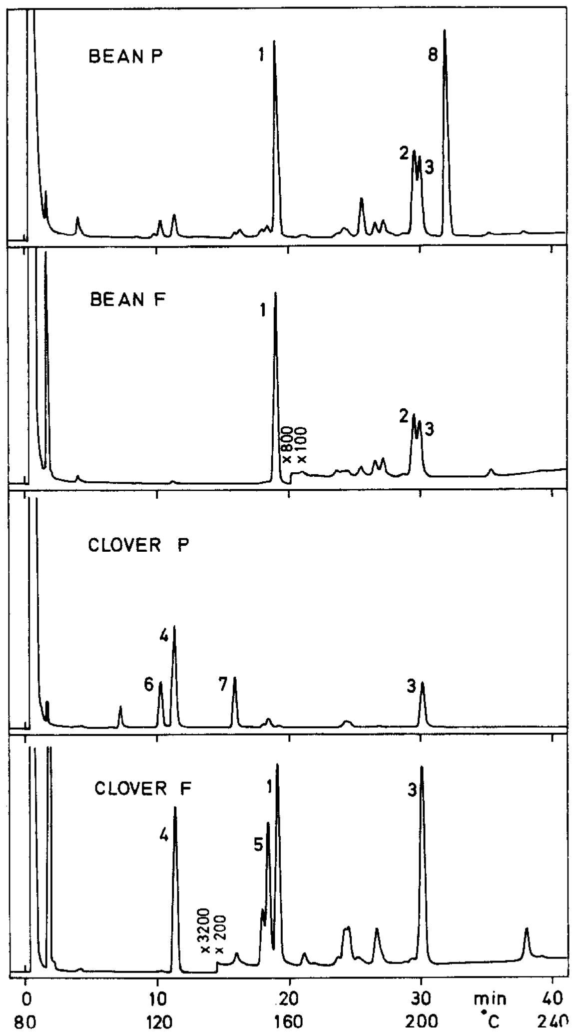

Fig. 1. GLC analysis of trimethylsilylated hydroxy acids isolated from bean and clover and preseparated into precipitate (P) and filtrate (F) fractions by treatment of the barium salts with 80% aqueous ethanol. 1. 2-*C*-Methyltetronic acid; 2. 2-*C*-(hydroxymethyl)pentonic acid; 3. gluconic acid; 4. glyceric acid; 5. threonic acid; 6. succinic acid; 7. malic acid; 8. inositol.

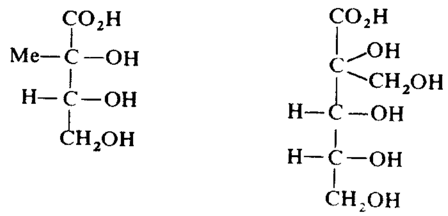

Fig. 2. Structures of the identified branched 2-*C*-methyltetronic and 2-*C*-(hydroxymethyl)pentonic acids.

may produce a different pattern of acids [7].

As illustrated by the lower part of Fig. 1, glyceric acid is the predominant acid obtained from clover. Gluconic acid, malic and succinic acids are the most prominent of the other compounds in 'clover P'. Threonic and gluconic acid, as well as the 2-*C*-methyltetronic acid, were identified in 'clover F' but the amounts are very small compared to the amount of glyceric acid which is recorded with a sixteen times higher attenuation.

The identical analytical conditions used gave virtually the same retention times for a given compound. This shows from Fig. 1 that some major identified compounds are present as minor components in other samples. The characteristic retention time of minor unidentified peaks in intermediate positions can be used for tentative structural deductions based on relationships between structure and retention [4].

DISCUSSION

A basic question is whether the identified hydroxy acids are present in the original plant material or formed during the isolation procedure. Monosaccharides are easily degraded and the procedure used [1] involves slightly alkaline treatments as well as acidic ion exchange steps. The usual branched acids might be expected to be the products of alkaline benzilic acid rearrangement of 1-deoxy-2,3-pentodiulose to give the 2-*C*-methyltetronic acid and of a 2,3-hexodiulose to give the 2-*C*-(hydroxymethyl)pentonic acid. Such rearrangements of dicarbonyl compounds may occur even at pH values *ca* 7.0 and at low temperatures [8]. The 1-deoxy-2,3-pentodiulose in turn might be formed from pentoses and pentuloses by rearrangements followed by β-elimination at C-1. The formation of 2-*C*-methyltetronic acids by this route on treatment of xylose with $Ca(OH)_2$ has been demonstrated [9].

Although formation of hydroxy acids by *in vitro* degradation of sugars may occur, much evidence is available for the present of a major part of the acids in the original plant material. This evidence includes metabolic studies by ^{14}C-labelling [10] and studies of differences in the composition of acids between various species [2] and even between varieties of one species [7]. The presence of sugar-related hydroxy acids has also been demonstrated in other species, e.g. in succulent plants [11].

In conclusion, the investigated group of carbohydrate-related hydroxy acids appears to be of great interest in studies of leguminous plants. It is evident that their presence in the living cell may have very important metabolic implications.

EXPERIMENTAL

The varieties studied were Golden Saxa for *Phaseolus vulgaris* and Red for *Trifolium incarnatum*. The isolation procedure involved as major steps (cf. [1]) extraction of the plant material with H_2O, neutralization with $Ba(OH)_2$, fractional precipitation of acids with aq. EtOH of increasing concentration and reprecipitation with $Ca(OH)_2$ after cation exchange. The detailed procedure is described elsewhere [2].

TMSi derivatives were prepared from the Ca salts by shaking with BSTFA + TMCS in Py [3]. Duplicate silylations after cation exchange and neutralization with NaOH to the more commonly used Na salts gave almost identical results. For GLC, a 3% OV-101 column (2 m × 2 mm) was used with linear temperature programming from 80 to 240° at 4°/min. The carrier gas was N_2 at 30 ml/min and detection was by FID. MS (EI) were recorded on a AEI Organic MS 20 GC–MS instrument with 70 eV electron energy.

Acknowledgements—The authors thank Mrs. Urszula Gulczynska (preparation of samples) and Mrs. Mary Mattsson (gas chromatography) for skilful technical assistance. The work was supported in part (for R. W. S. and B. T.) by the Polish Academy of Sciences, (PAN), within the project 946/V.

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