

inositol are higher in the tissues and fluids of foetal and growing animals than in those of adult animals^{7,8}. While it is possible that inositol, in virtue of the high concentration encountered, may play an important part in the carbohydrate metabolism of tissues concerned with rapid metabolism, it is also possible that growing tissues need stores or reserves of inositol, both in plants and in animals. The true significance of the high inositol concentrations cannot as yet be assessed.

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Occurrence of Shikimic Acid in Gooseberry Fruits

IN a recent survey of the organic acids of fruits, shikimic acid has been found in considerable amount in each of the varieties of gooseberry examined. Aqueous extracts of unripe fruits of the varieties Levellier, Careless and Whinham's Industry, after precipitation of pectin with 60 per cent acetone, were investigated by paper chromatography using the following three solvents: (1) benzyl alcohol - *iso*-propyl alcohol - *tert*-butyl alcohol - water - formic acid (3 : 1 : 1 : 1 : 2 per cent)¹, (2) *n*-propyl alcohol - conc. ammonia (70 : 30)², (3) phenol - water - formic acid (3 : 1 : 1 per cent)¹. In addition to malic and citric acid spots, a third spot (*A*) was observed with R_F values in solvents (2) and (3), 0.17 and 0.58 respectively, identical with those of shikimic acid; citric acid and shikimic acid do not separate in solvent (1). Spot *A* separated from quinic and dihydroshikimic acids in solvent (3)³, R_F values 0.58, 0.50 and 0.66 respectively, and rapidly gave a greenish-yellow colour at room temperature on spraying with sodium metaperiodate followed by sodium nitroprusside and piperazine⁴. This reagent is so far only known to give a reaction with quinic, shikimic and dihydroshikimic acids⁵. It has now been found that shikimic acid reacts at room temperature within a few minutes, while the other acids react more slowly. This observation is an additional aid to identification.

Addition of the gooseberry pectin-free extract to a silica gel column and elution with *tert*-butyl alcohol - chloroform mixtures of increasing *tert*-butyl alcohol content showed an acid peak in the same position as that shown by an authentic specimen of shikimic acid. Examination of the appropriate fractions by paper chromatography also indicated the presence of shikimic acid in amounts corresponding to 0.30 per cent of the fresh weight of the fruit.

In order to isolate the suspected shikimic acid, a volume of pectin-free extract was first treated with

'Amberlite' IR-120 resin in the hydrogen form, and passed through a column of 'Amberlite' IRA 400 (acetate) resin, which was then treated with 0.1 *N* acetic acid. Acid *A* was eluted at an early stage together with a trace of quinic acid (identified by the paper chromatographic methods described above). The two acids were separated on a silica gel column; acid *A* was eluted first. 0.15 gm. was obtained from 50 gm. of berries and, after treatment with charcoal, crystallized from 95 per cent ethanol and then from glacial acetic acid with melting point 184-85°, which showed no depression on admixture with an authentic specimen of shikimic acid. Elementary analysis gave C, 48.41; H, 5.90 per cent; shikimic acid requires C, 48.27; H, 5.79 per cent. Acid *A* is therefore shown to be shikimic acid and contributed in the gooseberry 20 per cent of the total acidity on a weight basis.

In contrast to the results of Hulme⁶, who observed increasing shikimic acid content of Bramley's Seedling with increasing maturity and the onset of senescence, the present results on the gooseberry variety Careless show that from the under-ripe stage to the fully ripe stage six weeks later, the weight of shikimic acid per berry remained practically constant.

The simultaneous appearance of shikimic and quinic acids in a plant tissue may be significant, although, in the bacterial mutants studied by Davis⁸, quinic acid was off the main route of aromatization from carbohydrate via shikimic acid to the aromatic amino-acids. The occurrence of quinic and shikimic acids in the same plant tissue was first observed by Boldt⁷, who isolated these acids from the carpels of *Illicium verum*, and later by Hulme and Richardson, who isolated them from grasses⁹ and by Hulme from apple fruits (Bramley's Seedling)⁵. Hathway¹⁰ has identified by paper chromatographic methods quinic and shikimic acids in myrobalans, and I have detected both acids in gooseberries, blackberries, cider apples, perry pears and quince fruits, using fractional elution from anion exchange resin columns and paper chromatography. The co-existence of these acids is probably extensive in plant tissues although their metabolic function is still unknown. L. F. Burroughs (private communication) has observed little difference in the pattern of free aromatic amino-acids of gooseberry and in soft fruits in which shikimic acid was either not detected or was found in trace amounts only. This, however, does not exclude differences in other aromatic constituents.

A detailed account of this work will be published elsewhere. I wish to thank Dr. A. Pollard, of this Station, for helpful advice and discussion.

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