Biosynthesis of α-Methylene-γ-butyrolactone, the Cyclized Aglycone of Tuliposide A

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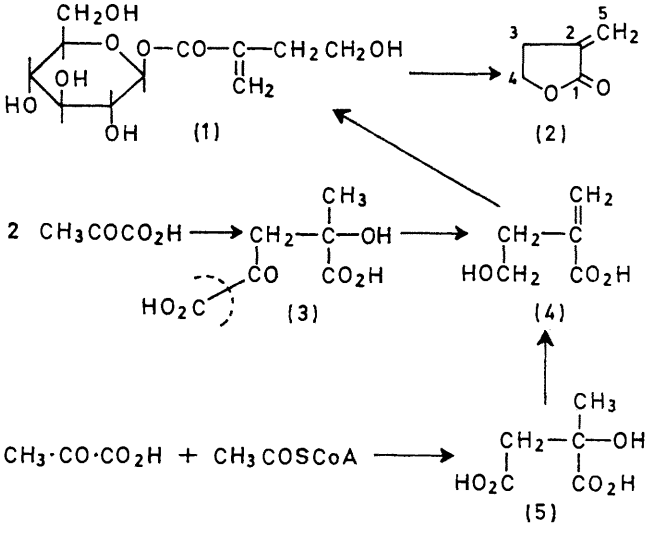
Summary The administration of [^{14}C]pyruvate to tulips resulted in the formation of labelled α-methylene-γ-butyrolactone, the pattern of labelling being consistent with the hypothesis that it is formed by a condensation between pyruvate and acetate.

TULIPOSIDE A (**1**) is a 1-acylglucoside,[1] found in tulips (*Tulipa gesneriana* L.), and on hydrolysis affords α-methylene-γ-butyrolactone (**2**), a substance which protects tulip bulbs from fungal attack.[2,3] A plausible biosynthetic route to the aglycone, γ-hydroxy-α-methylenebutyric acid (**4**), would involve an aldol condensation between two molecules of pyruvate yielding γ-methyl-γ-hydroxy-α-oxoglutaric acid (**3**).[4] Such a biosynthetic scheme has been suggested and partially substantiated for γ-methyl-γ-hydroxyglutamic acid[5] and γ-methyleneglutamic acid,[6] which are readily derivable from the α-keto-acid (**3**).

In separate experiments sodium [1, 2, and 3-^{14}C]pyruvate was fed to tulips (5 months from first appearance of sprouts, at the flowering stage) either by direct injection into the bulbs, or by cotton wicks inserted into the stems. The amount of radioactivity found in the α-methylene-γ-butyrolactone increased with time, for example the percentage absolute incorporation into the lactone after feeding [3-^{14}C]-pyruvate for 1·5 h, 3 h, 9 h, 1 day, and 9 days was 0·012, 0·036, 0·045, 0·155, and 0·79%, respectively. The location of activity in the lactone was determined as follows. Hydrogenation over platinum afforded α-methyl-γ-butyrolactone which was reduced with hydrogen iodide to 2-methylbutanoic acid. A Schmidt reaction on this acid yielded carbon dioxide [C-1] and 2-aminobutane which was oxidized with potassium permanganate to butan-2-one. Treatment of this ketone with sodium hypoiodite yielded iodoform [C-5] and propanoic acid which was further degraded by established methods.[7]



The lactone (**2**), obtained after feeding [1-^{14}-C]pyruvate was labelled almost exclusively at C-1, with less than 1% of activity on the other carbons. [2-^{14}C]Pyruvate afforded

the lactone labelled as follows, essentially the same distribution of activity being observed when the tulips were allowed to grow for 1, 3, and 9 days after feeding: C-1 (1·6—2%), C-2 (50—71%), C-3 (0·8—1·7%), C-4 (10—18%), and C-5 (0·7—0·8%). The lactone isolated 1 day after feeding [3-^{14}C]pyruvate was labelled thus: C-1 (0·8%), C-2 (3%), C-3 (3%), C-4 (5%), and C-5 (91%). Longer feeding times with this precursor reduced the amount of activity at C-5, but even after 10 days, 65% of the activity was located at C-5.

It is thus clear that C-1, -2, and -5 of γ-hydroxy-α-methylenebutyric acid are derived directly from pyruvate. However contrary to our expectations, pyruvate does not seem to be directly incorporated into C-3 and C-4. The results are consistent with the conversion of pyruvate into acetyl-coenzyme A which could then condense with pyruvate to yield citramalic acid (**5**); unexceptional reduction and dehydration would then afford (**4**). Thus [1-^{14}C]acetate would be formed from [2-^{14}C]pyruvate, resulting in labelling at C-4. On the other hand [3-^{14}C]pyruvate would yield methyl-labelled acetate and passage of this through the Krebs cycle would afford acetic acid labelled on both carbons, resulting in non-specific labelling of C-3 and C-4 of the lactone.

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