THE CONVERSION OF D-GLUCURONOLACTONE TO L-GULONIC ACID BY THE DETACHED RIPENING STRAWBERRY

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Received August 19, 1959

In the course of an investigation of the labeled metabolic products obtained from individual, detached, ripening strawberries which had been administered (1-^{14}C)- or (6-^{14}C)D-glucuronolactone through the cut stem, a very radioactive aldonic acid was isolated and tentatively identified as gulonic acid by its migration on paper in three different chromatographic solvents (Finkle, Kelly and Loewus, in press). We now wish to report the positive characterization of this reduction product of D-glucuronolactone as L-gulonic acid.

The radioactive acid, which appeared in the 70 per cent ethanol soluble fraction of the strawberry, was recovered by means of a dilute formic acid gradient from Dowex 1 (formate) as a composite peak referred to as "B" in the original experiments. The fractions corresponding to this peak were concentrated to a small volume and then streaked on large sheets of Whatman No. 1 paper. After thorough drying, the sheets were developed by descending chromatography in ethyl acetate-acetic acid-water (3:1:3, upper phase) (Rao, Beri and Rao, 1951) for 20 hours, dried and then stored against Kodak No-Screen X-ray film for 12 days in cassettes. The film revealed similar patterns of radioactive bands from both (1-^{14}C) and (6-^{14}C)D-glucuronolactone-labeled berries as follows: 25-30 mm. from the origin, faint darkening; 33-36, faint; 60-75, dark; 80-100, dark; 175-190, very faint; 200-220, dark; and 260-275, very faint. The band at 80-100 mm. (which overlapped the 60-75 mm. band slightly) corresponded to gulonic acid, the one at 200-220 mm. to

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gulonolactone. The 60-75 mm. band was not identified. Gulonolactone was formed from gulonic acid during the concentration of the peak "B" volume and removal of formic acid. The gulonolactone band was eluted with N acetic acid and to the eluate was added 70 mg. of pure L-gulono-γ-lactone prepared by the reduction of sodium D-glucuronate with $NaBH_4$ (Wolfrom and Anno, 1952). The solution was concentrated to dryness at 40°, *in vacuo*, and held *in vacuo* at 60° for an additional hour. The residue was taken up in hot glacial acetic acid, allowed to crystallize and then recrystallized from this solvent until there was no further change in activity (L-gulonolactone is soluble in glacial acetic acid to the extent of about 3 mg. per ml. at 25° and 15 to 20 mg. at 100°).

The recrystallized L-gulonolactone was further characterized by converting it to the amide (Hudson and Komatsu, 1919) and to the phenyl hydrazide (Fischer and Curtiss, 1892). It was degraded chemically with $NaIO_4$ as previously described for the degradation of sodium L-galactonate (Seegmiller, Axelrod, and McCready, 1955) to obtain C-1 as CO_2, C-(2+3+4+5) as HCOOH and C-6 as HCHO. The activities of the successive recrystallizations, the derivatives and the degraded products after conversion to CO_2 are listed in Table I. These values are the actual activities of the L-gulonolactone after dilution. The quantity of gulonic acid recovered from each berry by means of the ion exchange column was too small to assay chemically. Titration of all fractions from a single berry corresponding to composite peak "B" revealed less than one mg. of acidity as a six carbon aldonic acid. About 5 per cent of the administered label was recovered as L-gulonic acid. Assuming no endogenous dilution of the precursor or the product had occurred, the specific activity of the L-gulonolactone closely approximated that of the administered D-glucuronolactone although a precise comparison was not possible.

Practically no ^{14}C redistribution into carbons other than the one bearing the original label was observed. The over-all conversion involved a hydrolysis of the lactone ring of D-glucuronolactone and a reduction of C-1 of this compound. The enzymic process probably involves an initial hydrolysis of

Table I

Radioactivity of L-Gulonolactone, Derivatives and Degraded Products from Strawberries Labeled with (1-^{14}C)- or (6-^{14}C)D-Glucuronolactone

Compound	D-Glucuronolactone Administered	
	(1-^{14}C)	(6-^{14}C)
	mμc/mg carbon	
L-Gulonolactone, 1st cryst. (6 carbons) 2nd "	2.11 2.05	0.95
3rd "	2.05	
L-Gulonic acid amide (6 carbons)	2.03	0.90
L-Gulonic acid phenyl hydrazide (12 carbons)	1.12	0.51
Degraded L-gulonic acid		
C-1	0.003	5.78
C-(2+3+4+5)	0.007	0.007
C-6	12.38	0.008
Theory for all ^{14}C in a single carbon*	12.30	5.73

* Calculated on the basis of the activity of L-gulonolactone, 2nd crystallization.

the lactone, an isomerization to D-fructuronic acid (Wahba, Hickman, and Ashwell, 1956) and finally, a stereospecific reduction to L-gulonic acid. The recovery of L-gulonic acid as the free acid from the ethanol-soluble portion of strawberries administered D-glucuronolactone supports this view. Additional studies are planned which will elucidate the enzymic nature of this conversion in higher plants.

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