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Oxalic Acid Synthesis in Shoots of *Oxalis pes-caprae*

THE PRECURSORS OF GLYCOLLIC ACID AND GLYOXYLIC ACID

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Oxalis pes-caprae contains large amounts of oxalic acid, which appears to be formed predominantly from glyoxylic acid and glycollic acid; the origin of these latter compounds was not established (Millerd, Morton & Wells, 1963). To account for synthesis of oxalic acid in *Aspergillus niger*, Cleland & Johnson (1956) proposed that two molecules of ribose were converted into five molecules of glycolaldehyde, which were then oxidized to oxalic acid via glyoxylic acid and glycollic acid. Glycollic acid may also be derived from ribose in photosynthetic tissue (Griffith & Byerrum, 1959). It appeared likely, however, that glycollic acid in *Oxalis* arose by reduction of glyoxylic acid, which could be formed by cleavage of isocitric acid catalysed by isocitrate lyase. Although Carpenter & Beevers (1959) had found that this enzyme occurred only in those plant tissues that actively convert fat into carbohydrate, their study did not include plant tissues that contained a high concentration of oxalic acid. Such tissues would clearly be active in the synthesis of C_2 compounds (glyoxylic acid). This paper describes the results of studies to determine the relative contribution of [2-^{14}C]glucose, [1-^{14}C]ribose, [1,5-$^{14}C_2$]isocitric acid and of sodium [1-^{14}C]glycollate to oxalic acid synthesis in the white emergent shoots of bulbs of *Oxalis pes-caprae*.

MATERIALS

Plants. Bulbs of *Oxalis pes-caprae* were harvested, germinated in the dark and used as described by Millerd *et al.* (1963).

Chemicals. The following ^{14}C-labelled compounds, with the specific activities noted, were used: D-[2-^{14}C]glucose, 13·1 μc/mg.; D-[1-^{14}C]ribose, 13·0 μc/mg.; sodium [1-^{14}C]-glycollate, 19·4 μc/mg. (all from The Radiochemical Centre, Amersham, Bucks.); DL(+)-allo-[1,5-$^{14}C_2$]isocitric acid lactone, 34·6 μc/mg. (California Corp. for Biochemical Research). Phenol, toluene, 3-methylpropan-1-ol, pyridine and formic acid (British Drug Houses Ltd.) were A.R. grade. Butan-1-ol (Colonial Sugar Refineries Ltd.) and propionic acid (British Drug Houses Ltd.), both L.R. grade, were redistilled before use. 2,5-Diphenyloxazole and *p*-bis-2,5-phenyloxazylbenzene were A.R. scintillation grade [Nuclear Enterprises (G.B.) Ltd., Scotland].

METHODS

Hydrolysis of DL(+)-*allo*-[1,5-$^{14}C_2$]*isocitric acid lactone.* Three equivalents of KOH were added to the lactone and the solution (final vol., 0·21 ml.) was maintained on a steam bath for 15 min. and then cooled. The final pH of the solution was 5–6 (cf. Kornberg & Beevers, 1957).

Treatment of shoots with radioactive compounds. D-[2-^{14}C]-Glucose (50 μc) was dissolved in 0·3 ml. of water and 0·02ml. was applied to each shoot. D-[1-^{14}C]Ribose (50 μc) was dissolved in 0·18 ml. of water and 0·02 ml. was applied to each shoot. Sodium [1-^{14}C]glycollate (100 μc) was dissolved in 1·0 ml. of water and 0·025 ml. was applied to each shoot. Potassium DL(+)-allo-[1,5-$^{14}C_2$]isocitrate (0·21 ml., containing approx. 20 μc) was administered immediately after hydrolysis of the lactone; 0·025 ml. was applied to each shoot. Two shoots were used in each experiment. A narrow groove was cut along the length of each shoot (approx. 1 mm. × 1 mm.), the slice of tissue removed and the appropriate volume of ^{14}C-labelled material was introduced with a micropipette. The shoots were kept in the dark for 1 hr. or 6 hr. at room temperature and then detached and extracted as described by Millerd *et al.* (1963). The combined extracts from the shoots were then divided into non-ionic compounds (mainly sugars), amino acids and organic acids by chromatography with Dowex 50 (H^+ form)

and Dowex 2 (CO_3^- form) as described by Millerd *et al.* (1963), except that amino acids were eluted with aq. 4 N-NH_3 soln. (10–15 ml.) and organic acids with aq. 4 N-HCl (10–15 ml.). The relative distribution of ^{14}C between the three fractions was obtained by plating a known volume (0·02–0·05 ml.) of the appropriate solution in an infinitely thin film on copper planchets and drying under an infrared lamp; the radioactivity was determined with a Geiger–Müller end-window tube. Individual compounds containing ^{14}C were identified by paper chromatography and radioautography.

Paper chromatography and radioautography. Whatman no. 1 paper (46 cm. × 57 cm.) was washed as described by Connell, Dixon & Hanes (1955), except that the wash with calcium acetate was omitted. Amino acid and organic acid extracts were evaporated to dryness at 30° (external temperature) on a rotary film evaporator connected to an oil vacuum pump. The residues were dissolved in 1·0 ml. of 20% (v/v) ethanol. For amino acids, 0·2 ml. portions of the extracts were applied to chromatograms and for organic acids 0·05 ml. portions of the extract were used. Most compounds were separated adequately by two-dimensional chromatography with phenol saturated with water at 24° and butan-1-ol–propionic acid–water (47:22:31, by vol.) as described by Benson *et al.* (1950). However, glycine and serine were not well defined in this system when there was considerable radioactivity in either compound. Glycine and serine were therefore eluted from the chromatogram with water, concentrated *in vacuo* and separated by ascending paper chromatography with pyridine–water (4:1, v/v). Citric acid and isocitric acid, which were not well separated in the two-dimensional system described, were also eluted from chromatograms with water and separated by double development of paper chromatograms with a solvent described by Ladd & Nossal (1954) modified to contain butan-1-ol–3-methylpropan-1-ol–water–pyridine–98–100% formic acid (3:6:7:4:1, by vol.) (E. Holdsworth, personal communication).

Compounds containing radioisotope were detected by radioautography (see Millerd *et al.* 1963). Areas on chromatograms containing ^{14}C-labelled compounds were identified and cut out for quantitative estimation of ^{14}C content.

Determination of radioactivity. The radioactivity in all compounds containing ^{14}C separated by paper chromatography and located by radioautography was determined by a liquid-scintillation technique essentially as described by Wang & Jones (1959). Measurements were made at room temperature with type N 664A scintillation counters connected with type N530F scalers (Ekco Electronics Ltd.). The scintillation counters were equipped with EM 19514S photomultiplier tubes. Unit no. 1 operated at 1200v (high voltage) and 10v (discriminator bias). Unit no. 2 operated at 1450v (high voltage) and 15v (discriminator bias); both units were set with an amplifier gain × 100. The scintillator solution consisted of 3 g. of 2,5-diphenyloxazole and 0·2 g. of *p*-bis-2,5-diphenyloxazylbenzene dissolved in 1 l. of toluene. Compounds containing ^{14}C were counted on paper by placing disks of paper face down on the window of counting pots (50 ml. Quickfit pots painted with white enamel, with ground-glass tops, 4·5 cm. diam.) in 5·0 ml. of scintillator. Optical contact of the base of the pot with the top of the photomultiplier tube was made with silicone oil (100 CSTKS; Swift and Co., Sydney, N.S.W.). Preliminary experiments confirmed that the area of the spot to be counted and the volume of scintillator between 5 and 15 ml. made no difference to the observed counting rate (Bousquet & Christian, 1960). The efficiency of each unit was determined by pipetting standard, carrier-free sodium [^{14}C]-carbonate on to paper disks, drying and counting as described. The efficiency of unit no. 1 was 58% and of unit no. 2 was 54%. These figures compare favourably with 55% efficiency recorded by Wang & Jones (1959) for compounds that were insoluble in toluene. A standard spot was counted at regular intervals to ensure that the observed count rates from both units were strictly comparable (Stitch, 1959). A known volume (0·01 ml.) of each extract, as applied to chromatograms, was counted on paper disks so that the observed counts in specific compounds from chromatograms could be expressed as a percentage of the total ^{14}C activity applied.

RESULTS

In each experiment, ^{14}C activity administered to the shoots was calculated. After extraction of the shoot material with ethanol and with water the recovery of such activity was assessed. For 1 hr. reaction periods the recovery of the administered radioactivity was 99–100%; for 6 hr. periods it was 88–98% (Table 1). The relative distribution of ^{14}C between sugars, amino acids and organic acids is also shown in Table 1. The radioactivity in each fraction is expressed as a percentage of the radioactivity recovered in the appropriate extract from *Oxalis* shoots. The loss of ^{14}C activity as a result of chromatography with Dowex 50 (H^+ form) and Dowex 2 (CO_3^- form) was relatively small with [2-^{14}C]glucose and with [1-^{14}C]ribose. The percentage loss with [1,5-$^{14}C_2$]isocitric acid and with [1-^{14}C]glycollic acid [6–12%) was considerably greater (Table 1).

The distribution of ^{14}C activity among specific amino acids (Table 2) and among specific organic acids (Table 3) was also determined. The radioactivity in a specific compound was expressed initially as a percentage of the radioactivity applied to the appropriate chromatogram. The results in Tables 2 and 3 record the radioactivity in specific compounds as a percentage of the total radioactivity recovered in extracts from *Oxalis* shoots.

DISCUSSION

The technique for the administration of ^{14}C-labelled compounds to *Oxalis* shoots by applying such compounds to grooves cut in the shoots differs from the injection method described by Millerd *et al.* (1963). Although the trends in metabolic pathways presented here and in previous studies (Millerd *et al.* 1963) are similar, the percentage incorporation of ^{14}C from one compound into another is not directly comparable between the two sets of results.

Table 1. *Distribution of* ^{14}C *activity in sugars, amino acids and organic acids of* Oxalis *shoots*

White shoots of *Oxalis* bulbs were treated with various ^{14}C-labelled compounds; after 1 hr. and after 6 hr. the shoots were detached and extracted with ethanol and then with water as described in the text. Radioactivity recovered in each extract is expressed as a percentage of the total ^{14}C activity administered to the shoots. Compounds in the extracts were fractionated into sugars, amino acids and organic acids and the ^{14}C activity of each was determined. Radioactivity in each fraction is expressed as a percentage of the total activity recovered from the shoots.

Compound applied	Time (hr.)	^{14}C activity recovered in shoot extract (%)	Distribution of ^{14}C activity in shoot extracts (%)			^{14}C activity recovered from columns (%)
			Sugars	Amino acids	Organic acids	
[2-^{14}C]Glucose	1	99·1	93·5	0·6	2·8	97·8
	6	88·7	71·8	5·8	8·0	96·5
[1-^{14}C]Ribose	1	99·4	97·1	0·6	1·6	99·9
	6	94·3	84·9	3·3	5·4	99·3
[1,5-$^{14}C_2$]Isocitrate	1	100·6	—	2·6	86·3	88·4
	6	97·9	—	5·7	80·3	88·0
[1-^{14}C]Glycollate	1	100·3	—	3·3	90·4	93·4
	6	95·4	—	10·3	77·3	91·8

A comparison of the distribution of the isotope showed a greater incorporation of radioactivity into amino acids and organic acids from [2-^{14}C]-glucose than from [1-^{14}C]ribose (Table 1). This difference was more evident after exposure for 6 hr. The application of [1,5-$^{14}C_2$]isocitric acid for 1 hr. resulted in a greater incorporation of isotope into the amino acid pool than from incubation for 1 hr. with [2-^{14}C]glucose. After 6 hr. the incorporation was the same (5·8 and 5·7 % respectively, Table 1). It is evident that glycollic acid contributed significantly to the amino acid pool. When [1-^{14}C]glycollic acid was administered to *Oxalis* shoots, 3·3 % of the ^{14}C activity applied was located in the amino acid fraction within 1 hr.; 10·3 % of the activity was present in the amino acid pool after 6 hr. (Table 1). These figures represent the largest incorporation into amino acids from any of the ^{14}C compounds administered.

After administration of [2-^{14}C]glucose for 1 hr. and for 6 hr. most radioisotope in the amino acid fractions was located in glutamic acid, aspartic acid and alanine (Table 2). The same pattern of incorporation is evident in these amino acids from [1-^{14}C]ribose (Table 2).

As shown in Table 3, the incorporation of ^{14}C into organic acids from [2-^{14}C]glucose and [1-^{14}C]-ribose was somewhat similar. Initially, phosphate esters contained most ^{14}C; subsequently, glyceric acid and acids of the tricarboxylic acid cycle became labelled. The relatively greater incorporation of ^{14}C into glyceric acid from [2-^{14}C]glucose (0·52 and 1·75 %) rather than from [1-^{14}C]ribose (0·08 and 0·39 %) was reflected in generally higher amounts of ^{14}C in acids of the tricarboxylic acid cycle derived from [2-^{14}C]glucose. The amount of isotope in oxalic acid derived from [2-^{14}C]glucose was also higher (0·04 and 0·78 %) than that derived from [1-^{14}C]ribose (0·01 and 0·41 %). The formation of glycollic acid from ribose, demonstrated in photosynthetic tissue (Griffith & Byerrum, 1959), does not appear to be a significant pathway in white *Oxalis* shoots.

In the longer period more ^{14}C from both [2-^{14}C]-glucose and [1-^{14}C]ribose was incorporated into oxalic acid and acids of the tricarboxylic acid cycle, particularly into citric acid, isocitric acid and malic acid. This suggested that one or more of these acids may be involved in oxalic acid synthesis. Millerd *et al.* (1963) have shown that malic acid did not contribute to oxalic acid production in *Oxalis*.

The distribution of ^{14}C among organic acids derived from [1,5-$^{14}C_2$]isocitric acid (Table 3) shows that within 1 hr. some 12·5 % of the recovered ^{14}C activity has been incorporated into α-oxoglutaric acid and 6·4 % into succinic acid. From this experiment it is not possible to assess whether all the ^{14}C activity in succinic acid was derived from α-oxoglutaric acid or partly from isocitric acid by the action of isocitrate lyase. Since considerable radioisotope is found in oxalic acid (2·2 %) and glycollic acid (0·8 %) it seems likely that at least some isocitric acid was split into glyoxylic acid and succinic acid.

The percentage incorporation of ^{14}C into amino acids (Table 2) and into organic acids (Table 3) after treatment of *Oxalis* shoots with [1,5-$^{14}C_2$]-isocitric acid for 6 hr. showed similar trends as for the 1 hr. period.

The striking change in the distribution of ^{14}C in the 6 hr. period was the conversion of isocitric acid into citric acid, rather than into α-oxoglutaric acid or into glyoxylic acid. That the isotope incorpora-

Table 2. *Distribution of* ^{14}C *activity among amino acids from extracts of* Oxalis *shoots*

Compounds were administered to shoots and extracts prepared as described in the text and Table 1. Amino acids in shoot extracts were separated by paper chromatography and the amount of radioactivity in each compound was estimated as described in the text. Results are expressed as a percentage of the total ^{14}C activity recovered from *Oxalis* shoots. Where the radioactivity in a compound was not detected, the entry is shown thus: —; the lower limit of detection was approx. twice the background count, corresponding to approx. 0·001 % of the ^{14}C activity applied.

Compound applied	Time (hr.)	^{14}C activity in specific amino acids separated by paper chromatography (%)															^{14}C recovered from chromatograms (%)
		Origin*	Asp	Glu	Ser	Gly	Asp(NH_2)	Glu(NH_2)	Thr	Ala	β-Ala	Tyr	Pro	Phe	Val	Peptides	Unidentified	
[2-^{14}C]-Glucose	1	0·003	0·17	0·20	0·03	0·01	—	0·01	—	0·15	—	—	—	—	0·03	—	—	92·4
	6	0·08	0·73	1·50	0·40	0·10	—	0·28	0·04	0·61	—	0·02	0·06	—	0·65	—	0·69	88·4
[1-^{14}C]-Ribose	1	0·01	0·07	0·09	0·05	0·01	—	0·11	—	0·25	—	—	—	—	—	—	—	89·4
	6	0·13	0·72	0·87	0·16	0·07	—	0·06	0·13	0·68	—	0·22	—	0·19	—	—	0·06	98·7
[1,5-$^{14}C_2$]-Isocitrate	1	0·51	0·51	0·68	—	—	—	0·08	—	—	—	—	—	—	0·10	—	0·51	93·2
	6	0·06	1·34	2·32	0·01	—	—	0·48	0·02	0·10	0·06	0·10	—	—	0·33	—	0·68	97·1
[1-^{14}C]-Glycollate	1	0·02	0·09	0·17	1·55	0·81	—	0·03	—	0·05	—	—	—	—	0·05	0·08	0·18	91·8
	6	0·03	0·14	1·02	6·40	1·63	0·07	0·23	—	0·10	—	—	—	—	0·03	0·17	0·07	96·1

* Material remaining at origin after development of chromatograms.

Table 3. *Distribution of* ^{14}C *activity among organic acids from extracts of* Oxalis *shoots*

Compounds were administered to shoots and extracts prepared as described in the text and Table 1. Organic acids in shoot extracts were separated by paper chromatography and the amount of radioactivity in each compound was estimated as described in the text. Results are expressed as a percentage of the total ^{14}C activity recovered from *Oxalis* shoots. Where the radioactivity in a compound was not detected, the entry is shown thus: —; the lower limit of detection was approx. twice the background counts, corresponding to approx. 0·001 % of the ^{14}C activity applied.

Compound applied	Time (hr.)	^{14}C activity in specific organic acids separated by paper chromatography (%)														^{14}C recovered from chromatograms (%)
		Origin*	Phosphate esters	Glyceric acid	Citric acid	Isocitric acid	α-Oxoglutaric acid	Succinic acid	Malic acid	Fumaric acid	Phosphoglycollic acid†	Glycollic acid	Glyoxylic acid	Oxalic acid	Unidentified	
[2-^{14}C]Glucose	1	0·14	0·76	0·52	0·11	0·05	—	0·18	0·16	—	—	0·03	—	0·04	0·29	82·8
	6	0·40	1·83	1·75	0·86	0·40	0·23	0·25	0·55	0·09	—	0·08	—	0·78	0·24	93·4
[1-^{14}C]Ribose	1	0·06	0·27	0·08	0·03	0·01	—	—	0·03	—	—	0·02	—	0·01	0·74	86·9
	6	0·29	0·52	0·39	0·24	0·13	—	0·04	0·35	—	—	0·03	—	0·41	2·61	91·8
[1,5-$^{14}C_2$]Isocitrate	1	1·29	—	—	5·05	54·44	12·52	6·39	—	—	—	0·78	0·35	2·16	0·69	96·9
	6	1·93	—	—	34·03	15·19	6·58	4·42	1·12	0·08	—	0·96	0·48	4·10	1·57	89·0
[1-^{14}C]Glycollate	1	4·88	—	—	0·34	0·20	0·54	—	0·81	—	0·63	60·21	1·99	2·98	1·08	81·5
	6	1·86	—	—	1·19	0·82	0·54	0·93	0·85	0·54	0·93	30·77	4·56	20·03	0·85	82·6

* Material remaining at origin after development of chromatograms.

† Tentatively identified.

tion from [1,5-$^{14}C_2$]isocitric acid into oxalic acid was only doubled between the 1 hr. and 6 hr. incubation periods (Table 3) may be due to a number of factors. Since the [^{14}C]isocitric acid employed was not uniformly labelled, redistribution of the carbon atoms of this acid during the operation of the tricarboxylic acid cycle would decrease the frequency of ^{14}C being incorporated into glyoxylic acid and oxalic acid. Furthermore, it is well established that isocitrate-lyase activity may be repressed by intermediates of the tricarboxylic acid cycle (Kornberg, Gotto & Lund, 1958). Isocitrate lyase purified from yeast (Olsen, 1959) exhibited a marked decrease in activity with DL(+)-alloisocitric acid compared with L$_s$-isocitric acid. With DL(+)-alloisocitric acid at four times the concentration of L$_s$-isocitric acid the enzyme had only 20% of the activity. Thus the use of DL(+)-allo-[1,5-$^{14}C_2$]isocitric acid may have resulted in partial inhibition of isocitrate lyase in *Oxalis* shoots.

The accumulation of isotope in citric acid (34·0%) after administration of [1,5-$^{14}C_2$]isocitric acid for 6 hr. may be due to the reversal of aconitate hydratase (EC 4.2.1.3, formerly known as aconitase) or to the inhibition of this enzyme. Increased incorporation of ^{14}C into aspartic acid, glutamic acid (Table 2) and malic acid (Table 3) in the 6 hr. period suggests that the tricarboxylic acid cycle continued to function, and that inhibition of aconitate hydratase may have resulted from oxalomalic acid (Ruffo, Adinolfi, Budillon & Capobianco, 1962) formed non-enzymically from glyoxylic acid and oxaloacetic acid (Ruffo, Romano & Adinolfi, 1959). The failure to observe similar inhibition by glyoxylic acid derived from glycollic acid may be due to the removal of this compound (by oxidation) before the formation of sufficient oxalomalic acid at the active site of aconitate hydratase; glyoxylic acid derived from isocitric acid in the mitochondria would be near the active site of aconitate hydratase.

To compare the contribution of glycollic acid and of isocitric acid to oxalic acid biosynthesis, [1-^{14}C]-glycollic acid was administered to *Oxalis* shoots.

For both 1 hr. and 6 hr. periods the only amino acids to contain a significant amount of isotope were glycine and serine (Table 2). Amongst the organic acids, within 1 hr. glyoxylic acid contained 2·0% and oxalic acid 3·0% of the recovered activity (Table 3). The most notable feature of the ^{14}C distribution in the organic acids after 6 hr. (Table 3) is the large incorporation of isotope into oxalic acid (20·0%). Thus in 1 hr. the percentage incorporation of ^{14}C into oxalic acid from [1,5-$^{14}C_2$]isocitric acid and from [1-^{14}C]glycollic acid is comparable (2·2% and 3·0% respectively). However, after 6 hr. there was a much greater incorporation from [1-^{14}C]glycollic acid into oxalic acid.

Thus in non-photosynthetic *Oxalis* shoots, glyoxylic acid, which is oxidized to oxalic acid (Millerd *et al.* 1963), may be formed both from the oxidation of glycollic acid and from the cleavage of isocitric acid. It is likely, however, that glycollic acid is derived *in vivo* by reduction of glyoxylic acid.

SUMMARY

1. The relative contribution of [2-^{14}C]glucose, [1-^{14}C]ribose, [1,5-$^{14}C_2$]isocitric acid and [1-^{14}C]-glycollic acid to oxalic acid synthesis in *Oxalis* has been studied. Shoots were incubated for 1 and 6 hr. after the administration of each ^{14}C compound and the products containing radioisotope were extracted and separated by paper chromatography and located by radioautography.

2. The ^{14}C content of specific amino acids and of specific organic acids was determined by counting such compounds on paper by a liquid-scintillation technique.

3. The results show that isotope from [1,5-$^{14}C_2$]-isocitric acid and from [1-^{14}C]glycollic acid was incorporated into oxalic acid more efficiently than from [2-^{14}C]glucose or from [1-^{14}C]ribose. Contrary to results obtained with photosynthetic tissue, ribose did not contribute significantly to glycollic acid synthesis in white *Oxalis* shoots.

4. Within 1 hr. the percentage incorporation of radioisotope from [1,5-$^{14}C_2$]isocitric acid and from [1-^{14}C]glycollic acid into oxalic acid was comparable. After 6 hr. the contribution from [1-^{14}C]-glycollic acid was greater than that from [1,5-$^{14}C_2$]-isocitric acid. Glycollic acid also acted as an efficient procursor for the biosynthesis of serine.

5. Oxalic acid synthesis in white *Oxalis* shoots appears to be associated with the oxidation of glyoxylic acid which may arise from glycollic acid or from the cleavage of isocitric acid.

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Enzymic Synthesis of Oxalic Acid in *Oxalis pes-caprae*

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Oxalic acid is formed in white shoots of bulbs of *Oxalis pes-caprae* by the oxidation of glyoxylic acid (Millerd, Morton & Wells, 1963*a*); glyoxylic acid is also reduced to glycollic acid *in vivo*. The incorporation of ^{14}C from [^{14}C]isocitric acid into oxalic acid suggested that *Oxalis* shoots contained isocitrate lyase, which could catalyse the cleavage of isocitric acid, forming glyoxylic acid and succinic acid.

This paper describes studies with extracts from shoots of *Oxalis*. The extracts have been shown to contain isocitrate lyase (L$_s$-isocitrate glyoxylate-lyase, EC 4.1.3.1, formerly known as isocitritase) and other enzymes concerned with the metabolism of C_2 compounds in this plant. A brief account of some of this work has been published (Millerd, Morton & Wells, 1962).

MATERIALS

General chemicals. All inorganic reagents used were of A.R. grade. DL(+)-Alloisocitric acid (trisodium salt, 42% D form), GSH, sodium glyoxylate monohydrate and α-oxoglutaric acid were A.R. grade (Sigma Chemical Co.). CoA, cytochrome *c* (horse-heart type II, 65%), FMN and ATP were also products of Sigma Chemical Co. Semicarbazide–HCl, 2,4-dinitrophenylhydrazine (British Drug Houses Ltd.) and potassium oxalate (Judex Chemical and Pharmaceutical Co.) were A.R. grade. Glycollic acid (recrystallized before use), succinic acid (British Drug Houses Ltd.), cysteine–HCl (E. Merck and Co.) and trichloroacetic acid (May and Baker Ltd.) were L.R. grade.

DL-*Isocitric acid.* This was prepared from the lactone (A.R.; allo-free; Sigma Chemical Co.) as described by Kornberg & Beevers (1957).

Norit SX-2. This charcoal (Harrington Bros. Ltd.) was washed three times with 6N-HCl and subsequently with water until no chloride was detectable in the washings.

2,6-*Dichlorophenol-indophenol.* Approx. 2 g. of dye (British Drug Houses Ltd.) was dissolved in 80 ml. of N-HCl, shaken well with 20 ml. of diethyl ether and filtered through sintered glass. The ether layer was washed with water (2 × 50 ml.) and extracted with 100 ml. of 2% $NaHCO_3$. The blue aqueous layer was separated and 30 g. of NaCl was added to it. The precipitate was collected and washed with 30 ml. of 30% NaCl.

$NADH_2$. This was prepared from NAD (C. F. Boehringer und Soehne) by reduction with ethanol and alcohol dehydrogenase (Rafter & Colowick, 1955). On completion of the reaction, the mixture was placed in a boiling-water bath for 3 min., cooled rapidly in an ice bath and denatured protein was removed by centrifuging. The supernatant containing $NADH_2$ was stored at −15°.

$NADPH_2$. This was prepared from NADP (C. F. Boehringer und Soehne) by reduction with sodium isocitrate and isocitrate dehydrogenase (Evans & Nason, 1953). On completion of the reaction, the pH of the solution was adjusted to 9·0–9·5 with N-NaOH, placed in a boiling-water bath for 3 min., cooled and centrifuged. The supernatant containing $NADPH_2$ was stored at −15°.

Phosphate buffers. All phosphate buffers were prepared from disodium hydrogen orthophosphate (A.R., British Drug Houses Ltd.) and adjusted to the required pH with 2N-HCl.

^{14}C-*Labelled compounds.* Sodium [1-^{14}C]glycollate, specific activity 19·4 μc/mg., and glyoxylic acid monohydrate, specific activity 32·6 μc/mg., were obtained from The Radiochemical Centre, Amersham, Bucks.

Oxalis *extracts.* Bulbs of *O. pes-caprae* were treated as described by Millerd *et al.* (1963*a*) and the white shoots of the germinated bulbs were allowed to grow in the dark for 1–5 months. Shoots, usually 200 g., were then detached from the bulbs. All subsequent steps were carried out at 2°. The shoots were cut into small pieces (approx. 3 cm.) immediately before grinding in a pre-chilled glass mortar in 3 vol. of 0·2M-phosphate, pH 8·3. This extraction was carried out in stages so that approx. 30–35 g. of *Oxalis* shoots were extracted in 100 ml. of phosphate. Under these conditions the pH of the brei was kept just above pH 7·0 but a check was always made that the pH did not fall below this level from excess of oxalic acid released from the shoots. The green–brown brei was screened through