dehydration. The liberation of $^{14}CO_2$ when unlabelled glyoxylate and [1,3-$^{14}C_2$]acetoacetate were incubated together (Table 5) is proof that the decarboxylation of the initial product can occur at neutral pH, and explains the increase in $^{14}CO_2$ production by rat-liver slices in the presence of glyoxylate and [1-^{14}C]octanoate (Table 3).

The reaction between acetoacetate and glyoxylate probably has no physiological importance since the normal concentration of glyoxylate occurring *in vivo* is very low (e.g. in rat liver: 0·05 μmole/100 mg. dry wt.; Liang, 1962). However, the condensation reaction reported here is of interest as an explanation of the apparent antiketogenic action of glyoxylate and its metabolic precursors glycollate and glycolaldehyde.

SUMMARY

1. Glycolaldehyde, glycollate and glyoxylate, but not formate or glycine, decrease the amount of acetoacetate formed by rat-liver slices.

2. Incubation of glyoxylate and acetoacetate in neutral solution at 37° results in a stoicheiometric removal of the two acids and the reaction is accelerated by Mg^{2+} ions.

3. A product of this condensation reaction has been isolated and characterized as β-acetylacrylic acid (4-oxopent-2-enoic acid).

4. It is suggested that the apparent antiketogenic action of glyoxylate (and also of glycolaldehyde and glycollate) is due to its non-enzymic condensation with acetoacetate.

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The Isolation of γ-Hydroxyarginine, as its Lactone, from Seeds of *Vicia sativa*, and the Identification of γ-Hydroxyornithine as a Naturally Occurring Amino Acid

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The natural amino acid γ-hydroxyarginine was first isolated from the sea cucumber *Polycheira rufescens* by Fujita (1959) and subsequently from the sea anemone *Anthopleura japonica* Verrill by Makisumi (1961). Its occurrence in plants too has been reported by Bell & Tirimanna (1963*a*), who identified it in the seeds of 17 species of *Vicia*.

The present paper describes its isolation, as the lactone, from seeds of *Vicia sativa*, and the identification of γ-hydroxyornithine in other species.

EXPERIMENTAL

Methods

Chromatography. One-dimensional chromatograms were prepared by the descending technique on Whatman no. 1 paper. The chromatograms were developed with ethyl methyl ketone–propionic acid–water (2:1:2, by vol.), butan-1-ol–acetic acid–water (12:3:5, by vol.), butan-1-ol–pyridine–water (1:1:1, by vol.), lutidine (mixed 2,4- and 2,5-isomers)–water (11:5, v/v), and phenol–water

(4:1, w/v) in the presence of the vapour from aq. ammonia (sp.gr. 0·88) added to the tank. Chromatograms were developed for 17 hr. except where otherwise stated.

Ionophoresis. Ionophoresis was conducted on Whatman no. 1 paper in buffer solutions of pH 1·9 [formic acid (98–100%)-acetic acid–water (33:148:1819, by vol.)], of pH 3·6 [acetic acid–pyridine–water (10:1:190, by vol.)] and of pH 11·6 (sodium carbonate, 0·01 M). With the buffers of lower pH a horizontal method essentially that of Gross (1961) was used, a potential of 75 v/cm. being applied for 30 min. At pH 11·6 a hanging-strip method was used, 5 v/cm. being applied for 17 hr.

Development of colours. Chromatography and ionophoresis papers were dipped in ninhydrin reagent [0·2% (w/v) ninhydrin in 95% (v/v) acetone], the modified Ehrlich's reagent [1·0% (w/v) *p*-dimethylaminobenzaldehyde in acetone–10 N-hydrochloric acid (9:1, v/v)] of Smith (1953) and the modified Sakaguchi's reagent [0·1% (v/v) 8-hydroxyquinoline in acetone followed by 0·2% (v/v) bromine in 0·5 N-sodium hydroxide] of Jepson & Smith (1953). Ninhydrin colours were developed at 80°.

Detection of γ-hydroxyarginine. γ-Hydroxyarginine was detected as a ninhydrin-positive compound giving a scarlet colour with Sakaguchi's reagent which was distinguishable from the orange–red colour given by arginine. These two amino acids could also be readily separated by paper chromatography with the ethyl methyl ketone–propionic acid–water, butan-1-ol–acetic acid–water and phenol–water solvents.

Isolation of γ-hydroxyarginine lactone

Finely ground seed of *Vicia sativa* (1600 g.) was extracted with acetone for 24 hr. and with methanol for 48 hr. in a modified Soxhlet apparatus. The defatted seed was then shaken for 2 hr. with 1·5 l. of 25% (v/v) ethanol and the suspension filtered after 17 hr. at room temperature. The residue was again extracted in the same manner and the combined extracts were concentrated to 1 l. under reduced pressure. The concentrate was passed through a column (3 cm. × 80 cm.) of De-Acidite FF, a strong anion-exchange resin, which was then washed with water until the effluent was neutral and gave no reaction with ninhydrin. The alkaline effluent and combined washings were passed through a column (3 cm. × 50 cm.) of Zeo-Karb 226, a weak cation-exchange resin, which was washed as described above for the De-Acidite FF column.

The basic amino acids were eluted from the Zeo-Karb 226 resin with N-ammonia, and the fractions giving a positive reaction with Sakaguchi's reagent were combined and concentrated *in vacuo* at room temperature over $CaCl_2$ and H_2SO_4. The viscous residue was dissolved in water (10 ml.) and the solution acidified to pH 4 by the dropwise addition of 11 N-HCl. The acidified solution was stirred with activated charcoal, filtered and concentrated to 5 ml. at 100°. On adding excess of ethanol to the concentrate an oil separated; after 17 hr. at 4°, the ethanol was decanted and the oil stirred in warm fresh ethanol to which water was added dropwise until solution was complete. On standing at 4° colourless needles separated from the solution and were recrystallized from aq. ethanol in the same way. The purified material (57 mg.) had m.p. 158° (uncorr.) (Found: C, 27·7; H, 6·2; Cl, 26·4; N, 20·4; loss at 110°, 7 ± 1. Calc. for $C_6H_{12}N_4O_2,2HCl,H_2O$: C, 27·4; H, 6·1; Cl, 26·6; N, 21·3; H_2O, 6·8%). This compound (artifact), which was more basic than the original amino acid (as indicated by its ionic mobility), gave the same scarlet reaction with Sakaguchi's reagent but a yellow and not a purple colour with ninhydrin. When dissolved in 0·1 N-NaOH the artifact changed (within 24 hr. at room temperature) to a less basic compound giving a purple reaction with ninhydrin and showing R_F values in five solvent systems and ionic mobilities at pH 1·9 and 3·6 identical with those both of the unknown guanidino compound in the seed and authentic γ-hydroxyarginine (kindly supplied by Dr Y. Fujita) which was used as a 'marker'. The amino acid itself behaved as a mono-α-amino carboxylic acid when treated successively with cupric nitrate and ninhydrin (Larsen & Kjaer, 1960). The infrared-absorption and nuclear-magnetic-resonance spectra of the artifact (Hirst & Foster, 1964) were consistent with this compound being the lactone of γ-hydroxyarginine.

Alkaline hydrolysis of the lactone

The isolated hydrochloride (5 mg.) was refluxed with 0·1 N-NaOH (5 ml.) for 3 hr. The solution was evaporated to dryness at room temperature under reduced pressure, redissolved in 2 ml. of 10% (v/v) propan-2-ol and brought to pH 7 with N-HCl. Spots (10 μl.) of the solution were chromatographed and subjected to ionophoresis on paper in the solvent systems and the buffer solutions described above. Each spot was run with a 'marker' of authentic γ-hydroxyornithine (supplied by Dr Y. Fujita) and the papers were developed with ninhydrin and Ehrlich's reagent.

The hydrolysate gave a strong ninhydrin-positive spot that corresponded on all papers to γ-hydroxyornithine and a fainter spot that also gave a yellow colour with Ehrlich's reagent.

Identification of γ-hydroxyornithine on paper. When developed successively with ninhydrin and Ehrlich's reagent as in the multiple-dipping sequence of Jepson & Smith (1953) the initial purple spots with ninhydrin given on paper by γ-hydroxyornithine and by the principal product of alkaline hydrolysis of the isolated artifact changed to stable brown–grey spots. Hydroxyproline (Jepson & Smith, 1953), 4-methyleneproline (Gray & Fowden, 1962) and homocitrulline (Bell, 1962) are reported to show colour changes with these reagents but only γ-hydroxyornithine appears to give the brown–grey colour.

When dipped in the periodate–acetylacetone reagent developed by Schwartz (1958) for the identification of serine and δ-hydroxylysine, the product of alkaline hydrolysis and the authentic γ-hydroxyornithine gave identical yellow spots, indicating that periodate oxidation had liberated formaldehyde and confirming the presence of amino and hydroxyl groups on the ultimate and penultimate carbon atoms of the chain respectively.

γ-Hydroxyornithine in Vicia

Finely ground seed (200 mg.) from each of 64 species of *Vicia* was stirred with ethanol (1 ml.) and 0·1 N-hydrochloric acid (1 ml.). After 17 hr. at room temperature the suspensions were centrifuged and the supernatants analysed by paper chromatography and ionophoresis on Whatman no. 1 paper in the solvent systems and buffer solutions described above.

The extracts from the seeds of *V. unijuga* and *V. onobrychoides* contained high concentrations (about 1%) of a

ninhydrin-positive compound with R_F values and ionic mobilities corresponding to those of authentic γ-hydroxyornithine in the five solvent systems and three buffer solutions used.

This compound gave the characteristic brown–grey reaction of γ-hydroxyornithine on paper when treated successively with ninhydrin and Ehrlich's reagent and a yellow colour with the periodate–acetylacetone reagent of Schwartz.

DISCUSSION

The genus *Lathyrus* may be subdivided into groups of species, each of which is characterized by a common association of free amino acids or related compounds that occur in concentrations of about 1% in the seeds of its constituent members (Bell, 1962). Among these compounds are αγ-diaminobutyric acid (Ressler, Redstone & Erenberg, 1961), homoarginine, lathyrine, γ-hydroxyhomoarginine (Bell, 1963) and a compound tentatively identified as β-oxalylaminoalanine (α-amino-β-oxamoylaminopropionic acid) (Adiga, Rao & Sarma, 1963). A survey of the ninhydrin-positive compounds present in the seeds of the closely related genus *Vicia* (Bell & Tirimanna, 1963*b*) has revealed that this genus may also be subdivided on the same basis. The compounds present in high concentration in the seeds of *Vicia* are not, however, the same as those in *Lathyrus*, and include arginine, canavanine, β-cyanoalanine (Ressler, 1962) and γ-hydroxyarginine, the isolation of which has been described in the present paper. Therefore one genus (*Vicia*) stores relatively high concentrations of canavanine and the C_6 guanidino amino acids arginine and γ-hydroxyarginine, whereas the other (*Lathyrus*) stores predominently the C_7 amino acids homoarginine, γ-hydroxyhomoarginine and the related compound lathyrine.

SUMMARY

1. The occurrence of γ-hydroxyarginine in plants has been confirmed by the isolation of its lactone from seeds of *Vicia sativa*.

2. A new natural amino acid, γ-hydroxyornithine, has been identified by chromatography and ionophoresis in the seeds of *Vicia onobrychoides* and *V. unijuga*.

3. The closely related genera, *Lathyrus* and *Vicia*, characteristically store different groups of guanidino amino acids.

4. A colour reaction for the identification of γ-hydroxyornithine on paper is described.

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The Isolation of γ-Hydroxyhomoarginine, as its Lactone, from Seeds of *Lathyrus tingitanus*, its Biosynthesis and Distribution

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A study of the free amino acids and related compounds in the seeds of 49 species of *Lathyrus* (Bell, 1962*a*) revealed the presence in the seeds of *L. aphaca*, *L. cyanus*, *L. sphaericus* and *L. tingitanus* of lathyrine (i.e. 2-aminopyrimidin-4-ylalanine; Bell & Foster, 1962), homoarginine (Bell, 1962*b*) and a third new amino acid designated B_3, subsequently reported to be γ-hydroxyhomoarginine (i.e. α-amino-ε-guanidino-γ-hydroxyhexanoic acid; Bell, 1963). The existence of the last-named amino acid had been predicted by Rao, Ramachandran & Adiga (1963), who visualized it as a likely inter-