SUMMARY

1. After administration of 300 mg. of corticosterone or cortisol to a man, the greater part of these two corticosteroids were found in the urine in ester sulphate fraction.

2. Evidence that sulphate was attached to C-21 was provided by the similarity of chromatographic behaviour with that of authentic corticosterone 21-sulphate or cortisol 21-sulphate, electrophoretic separation for cortisol sulphate and the reaction to tetrazolium being positive only after hydrolysis.

We are indebted to 'Expandia' and 'Société Normande d'Extraction et de Synthèse' and to 'Laboratoire Roussel' for the generous supplies of corticosterone and cortisol respectively.

REFERENCES

- Burma, D. P. (1953). *Analyt. chim. acta*, **9**, 513.
 Burton, R. B., Zaffaroni, A. & Keutman, E. H. (1951). *J. biol. Chem.* **188**, 763.
 Bush, I. E. (1952). *Biochem. J.* **50**, 370.
 Cavina, G. & Tentori, L. (1958). *Clin. chim. Acta*, **3**, 160.
 Cohen, S. L. & Oneson, I. B. (1953). *J. biol. Chem.* **204**, 245.
 Crepy, O., Jayle, M. F. & Meslin, F. (1957). *Acta Endocr., Kbh.*, **233**, 24.
 Crepy, O. & Judas, O. (1960). *Rev. franc. Étud. clin. biol.* **5**, 284.
 Edwards, R. W. H., Kellie, A. E. & Wade, A. P. (1953). *Mem. Soc. Endocrin.* **2**, 53.
 Grassmann, W. & Hannig, K. (1952). *Hoppe-Seyl. Z.* **290**, 1.
 Jarrige, P. & Lafoscade, G. (1959). *Bull. Soc. Chim. biol., Paris*, **41**, 1197.
 Neher, R. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, **4**, 128.
 Pasqualini, J. R. (1958). *C.R. Acad. Sci., Paris*, **246**, 2945.
 Pasqualini, J. R. (1960). *C.R. Acad. Sci., Paris*, **250**, 1929.
 Pasqualini, J. R., Zelnik, R. & Jayle, M. F. (1960). *Experientia*, **16**, 317.
 Peterson, R. E. & Pierce, C. E. (1960). *J. clin. Invest.* **39**, 741.
 Peterson, R. E., Wyngaarden, J. B., Guerra, S. L., Brodie, B. B. & Bunin, J. J. (1955). *J. clin. Invest.* **34**, 1779.
 Schneider, J. J. & Lewbart, M. L. (1956). *J. biol. Chem.* **222**, 787.
 Vlitos, A. J. (1953). *Contr. Boyce Thompson Inst.* **17**, 127.
 Zaffaroni, A. (1953). *Recent Progr. Hormone Res.* **8**, 51.

Biochem. J. (1961) **81**, 149

The Biosynthesis and Metabolism of Polyols

SORBITOL (D-GLUCITOL) OF PLUM LEAVES

By J. D. ANDERSON, P. ANDREWS* AND L. HOUGH

Department of Organic Chemistry, University of Bristol

(Received 27 February 1961)

Acyclic polyhydric alcohols occur in many species of plants (Barker, 1955), although in the majority of cases neither quantitative data on the amounts present nor information on their metabolic role is available. Hypotheses have been advanced to explain the metabolism of polyols with little supporting evidence. Thus Donen (1939) suggested that sorbitol is formed in plum fruit when the concentration of hexoses reaches a critical level, whereas Kidd, West, Griffiths & Potter (1938) found that much of the sorbitol in pears is replaced by hexulose (probably fructose) as the fruit ripens. Sorbitol can also be utilized for polysaccharide synthesis in the leaves of Rosaceae and Gramineae (Barker, 1955; Altermatt & Neish, 1956), but nothing is known of the extent of this conversion under natural conditions.

Experiments with $^{14}\text{CO}_2$ originally designed to yield information on the biosynthesis of leaf poly-

saccharides (Andrews & Hough, 1958) indicated that sorbitol, the acyclic polyol in plum leaves, has an important role in the metabolism of these leaves. The incorporation of ^{14}C into sorbitol was considerably greater than into starch, and this observation initiated further studies of sorbitol metabolism in plum leaves.

Adcock (1957) assayed sorbitol by oxidation with sodium periodate and subsequent estimation of the two molecular equivalents of formaldehyde produced from the primary alcohol groups (O'Dea & Gibbons, 1953). Before oxidation, interfering substances were degraded by heating in sodium carbonate solution, and the degradation products removed by treatment with ion-exchange resins. However, when applied to plum-leaf extracts Adcock's method gave inconsistent results, a conclusion supported by White (1958), who suggested a preliminary acid hydrolysis of non-reducing carbohydrates such as sucrose to facilitate their degradation and removal. A simpler method

* Present address: National Institute for Research in Dairying, Shinfield, Reading, Berks.

for removing interfering substances was devised, in which the acid-hydrolysis step and ensuing alkaline degradation were effected in one process with ion-exchange resins. The modified assay procedure was used to estimate sorbitol in plum-leaf extracts.

A preliminary account of this work has been published (Anderson, Andrews & Hough, 1959).

MATERIALS AND METHODS

Experiments with $^{14}\text{CO}_2$

Radioactivity measurements. Compounds containing ^{14}C were burnt in a stream of O_2 in a micro-analytical combustion apparatus (F. J. McMurray, Dartford, Kent) and the $^{14}\text{CO}_2$ so produced was passed into hot $\text{Ba}(\text{OH})_2$ solution (saturated at 20°). The precipitated $\text{Ba}^{14}\text{CO}_3$ was collected with suction on filter-paper circles (2.8 cm. diam.) as uniform disks (2 cm. diam.; wt. about 20 mg./cm.², equivalent to infinite thickness for counting), and washed with water, mounted on circular brass plates and dried at 120° .

Radioactivity was determined in a counter with a thin end-window Geiger tube (Twentieth Century Electronics Ltd., type EW. 3H); sufficient counts were taken to give a standard error of not more than $\pm 3\%$. The counting equipment was calibrated in terms of $\text{Ba}^{14}\text{CO}_3$ by determining the count rate of 'infinitely thick' layers of $\text{Ba}^{14}\text{CO}_3$ prepared from poly(^{14}C methyl methacrylate) of known specific activity, and a disk of this polymer was used as a standard reference source.

Labelled compounds were obtained from The Radiochemical Centre, Amersham, Bucks.

Incorporation of $^{14}\text{CO}_2$ into plum leaves. Spurs bearing four or five leaves, 7–10 cm. long, were removed from a plum tree (*Prunus domestica* var. Victoria) during a morning in early September, and the cut ends of the stems were immersed in water. After being kept in the dark for 24 hr. the spurs were exposed to light in the presence of $^{14}\text{CO}_2$. In each experiment about 20 leaves were used, sufficient to give about 2 g. of material soluble in aqueous methanol. The spurs were placed in a 4 l. desiccator, and $^{14}\text{CO}_2$ was generated *in situ* by adding lactic acid (80%, v/v, in water) to $\text{Ba}^{14}\text{CO}_3$ (about 1.8 g.; specific activity from 7.2 to $16.0 \mu\text{C}/\text{m-mole}$ in different experiments), sufficient to give an initial $^{14}\text{CO}_2$ concentration of 5%, v/v. The leaves received a steady illumination of about 400 ft.-candles and were allowed to incorporate and metabolize the $^{14}\text{CO}_2$ in the desiccator for periods of 1.5–8 hr. in different experiments. In experiments of longer duration the spurs were removed from the desiccator after the 8 hr. period and kept in a gentle current of air under approximately the same illumination, since trial experiments had shown that all the $^{14}\text{CO}_2$ supplied to the leaves was incorporated within 8 hr. For further details see Andrews & Hough (1958).

Isolation and characterization of [^{14}C]sorbitol from plum leaves. After photosynthesis the leaf laminae were quickly broken up, immediately dropped into boiling ethanol and extracted exhaustively with methanol in a Soxhlet apparatus. The combined ethanol and methanol extracts were submitted to paper chromatography, with butan-1-ol-ethanol-water (40:11:19, by vol.), butan-1-ol-pyridine-water (10:3:3, by vol.) or butan-2-one-acetic acid-saturated aq. boric acid (9:1:1, by vol.) (Rees & Reynolds,

1958) as mobile phase, and $\text{AgNO}_3\text{-NaOH}$ (Trevelyan, Procter & Harrison, 1950) or *p*-anisidine hydrochloride (Hough, Jones & Wadman, 1950) as spray reagent. Appropriate areas of the chromatograms were cut into 0.5 in. squares, which were examined for ^{14}C activity in the thin end-window counter.

[^{14}C]Sorbitol was isolated from alcoholic extracts as follows. After evaporation of the alcohol, the residues were suspended in water and treated with Bio-Deminrolit (The Permutit Co. Ltd., London, W. 4) mixed-bed ion-exchange resin (about 25 g./g. of dry alcohol extract) for 6–8 hr. at $90\text{--}100^\circ$. The neutral supernatant and aqueous washings of the resin were combined, concentrated to dryness and the residual syrups were dried over phosphoric oxide and weighed. Paper-chromatographic examination indicated that these syrups consisted mainly of sorbitol, so their weights were used in calculating the approximate amount of ^{14}C in sorbitol.

Portions of these syrups were submitted to chromatography on large sheets of Whatman no. 1 paper for 2 days, with butan-1-ol-pyridine-water (10:3:3, by vol.) as mobile phase. Marker strips were then sprayed with the $\text{AgNO}_3\text{-NaOH}$ reagent to detect sorbitol, and the appropriate regions of the unsprayed paper were cut out and eluted with water. The eluates were evaporated to dryness and the residues acetylated by treatment overnight at room temperature with excess of acetic anhydride in a large excess of anhydrous pyridine (Verley & Bölsing, 1901). Water and chloroform were added, then the organic phase was separated, washed successively with 2N-HCl, saturated NaHCO_3 solution and water, dried with CaCl_2 , decolorized with charcoal and evaporated. The reaction products crystallized, and recrystallization from ethanol gave [^{14}C]sorbitol hexa-acetate with m.p. $98\text{--}99^\circ$ in each case, undepressed on admixture with an authentic sample. This derivative was used to determine the specific activity of [^{14}C]sorbitol from the photosynthesis experiments; further recrystallizations produced no change in specific activity.

[^{14}C]Sorbitol from its hexa-acetate. Crystalline [^{14}C]sorbitol hexa-acetate (250 mg.) was dissolved in dry chloroform (25 ml.) and a solution of sodium methoxide in methanol (5%, w/v; 3 ml.) added. After 2 hr. at 20° , the precipitated [^{14}C]sorbitol was centrifuged off and dissolved in water, then the solution was treated with Amberlite IR-120(H) resin to remove traces of Na^+ ions and evaporated to yield [^{14}C]sorbitol as a colourless glassy solid.

Isotopic-dilution analysis for sorbitol in a plum-leaf extract. [^{14}C]Sorbitol (103.4 mg.), which on combustion had given $\text{Ba}^{14}\text{CO}_3$ with a specific activity of 499 counts/min. at infinite thickness under standard conditions, was dissolved in a portion (250 ml.) of an alcoholic extract of non-radioactive plum leaves (≈ 3.75 g. dry wt.). After evaporation of the solvent, crystalline sorbitol hexa-acetate was prepared from the residue. After recrystallization this derivative gave $\text{Ba}^{14}\text{CO}_3$ with a specific activity of 62.7 counts/min. at infinite thickness under the same conditions as before.

Isolation of [^{14}C]glucose from plum-leaf starch. The alcohol-insoluble leaf residues from each photosynthesis experiment were dried, weighed, assayed for ^{14}C and then extracted successively with hot water, cold N-NaOH and hot 2.5N-NaOH. The three fractions so obtained from each batch of leaves were hydrolysed separately ($\text{N-H}_2\text{SO}_4$; 8 hr. at 100°) and the resultant monosaccharides were

separated by paper chromatography with butan-1-ol-ethanol-water (10:3:3, by vol.) as mobile phase. Glucose was eluted with water from the appropriate sections of the chromatograms, then the eluates were evaporated under reduced pressure, the residues were redissolved in water and the resultant solutions were passed through columns of Amberlite IR-120(H) and IRA-400(CO₂) resins and finally evaporated to dryness. The resultant syrups, after paper-chromatographic examination as a check on purity, were dried over phosphoric oxide and weighed, then inactive D-glucose was added and the sugars were crystallized and assayed for ¹⁴C activity.

Quantitative estimation of sorbitol

Sodium metaperiodate reagent. The grade described as 'for glycerol determinations' (Hopkin and Williams Ltd.) was used. A 0.015M solution was prepared as required by diluting a stock 0.3M solution, which was stored in the dark in a stoppered flask. All manipulations involving periodate solutions were carried out in as dim a light as was consistent with accuracy.

Sodium periodate-sodium hydrogen carbonate solution. Equal volumes of 0.015M-sodium metaperiodate and a freshly prepared saturated (at 20°) solution of NaHCO₃ were mixed immediately before use.

Lead dithionate solution. Barium dithionate was converted into the lead salt by the method of O'Dea & Gibbons (1953), giving an aq. 15% (w/v) solution, which was stored at 0°.

Chromotropic acid reagent. Chromotropic acid specially prepared 'for formaldehyde determinations' (Hopkin and Williams Ltd.) was dissolved in H₂SO₄ as described by O'Dea & Gibbons (1953) and the solution was stored in the dark.

Periodate method for sorbitol estimation. The periodate-oxidation reaction was performed in tapered 10 ml. centrifuge tubes, by adding sodium periodate-NaHCO₃ soln. (1 ml.) to the solution (2 ml.) for assay, containing not more than 200 µg. of sorbitol. After 1 hr., excess of periodate was destroyed by adding lead dithionate solution (1 ml.), then the precipitated lead salts were moved by centrifuging and a portion (1 ml.) of the clear supernatant was added to chromotropic acid reagent (10 ml.) contained in a 50 ml. centrifuge tube. Precipitated lead sulphate was removed on the centrifuge and the clear supernatant was heated in a stoppered tube in a darkened, vigorously boiling-water bath for exactly 30 min. Thereupon the tube was cooled to room temperature in running tap water and the light-absorption of the solution measured in an EEL colorimeter with a no. 626 filter. Standard curves were prepared for the range 15-200 µg. of sorbitol in 2 ml. of solution. Although deviations from linearity were noted in the upper part of the calibration curve, where the periodate concentration becomes limiting, reproducible results were obtained with an accuracy of ±3%.

Estimation of sorbitol in the presence of sucrose. Sorbitol (0.4-2.2 g.) and sucrose (about 0.5 g.) were dissolved in water (50 ml.) and the solutions were diluted to 1 l. with ethanol. Portions (10 ml.) were transferred to 50 ml. conical flasks and the solvents evaporated on a boiling-water bath. Amberlite IR-120(H) (3 g.) and IR-400(OH) (10 g.) resins were added with sufficient water to cover them, and heating was continued with addition of more water as necessary. After 6 hr. the contents of the flasks,

including the resins, were washed into glass columns (1 cm. × 35 cm.), each containing about 10 ml. of a mixture of equal parts of Amberlite IR-120(H) and IR-400(OH) resins. Complete elution of the sorbitol was effected by passing water (230 ml.) through the columns. The effluents were collected in graduated flasks, diluted to 250 ml. with water and portions (2 ml.) taken for estimation of sorbitol by the periodate method as described above.

Estimation of sorbitol added to plum-leaf extracts. Sorbitol (10-100 mg.) was added to portions (50 ml., containing roughly 30 mg. of sorbitol) of the alcoholic extracts of two batches of plum leaves. The alcohol was evaporated, the residues and added sorbitol were dissolved in water and suitable portions used for estimation of sorbitol as described above for sorbitol-sucrose mixtures.

Sorbitol content of plum leaves during 20 hr. Three plum trees (var. Victoria) in an orchard at Long Ashton, Somerset, were selected for this experiment. During warm, dry weather in July, five leaves were removed randomly from each tree at the intervals of time shown in Table 5. The leaf laminae were broken up and plunged into boiling ethanol (50 ml.) immediately after picking. The insoluble material was filtered off and extracted with methanol in a Soxhlet apparatus for 2 days. The final alcohol-insoluble residues were dried, at first at 60° and then over phosphoric oxide *in vacuo*, and weighed. The combined extracts from each group of 15 leaves were diluted to 500 ml. Samples (10 ml.) were evaporated, the residues treated with ion-exchange resins as described above, and the final solutions were used for sorbitol estimations by the periodate method.

RESULTS AND DISCUSSION

An investigation of polysaccharide metabolism in plum leaves with ¹⁴CO₂ (Andrews & Hough, 1958) was extended by studying, at the same time, the incorporation of ¹⁴C into the alcohol-soluble fraction of the leaves. Mature Victoria-plum leaves, which had been kept in the dark for 24 hr. to deplete their starch content, were allowed to photosynthesize in an atmosphere containing initially 5% (v/v) of ¹⁴CO₂; then they were extracted exhaustively with methanol, and the extracts were submitted to paper chromatography. When the chromatograms were monitored for ¹⁴C activity, a major part of the activity was located in the main polyol constituent of the leaves, after periods of photosynthesis from 1.5 to 24 hr.

This polyol was isolated and characterized as sorbitol (D-glucitol). No other hexitol or acyclic polyol was found in plum-leaf extracts by paper chromatography in three solvents capable of distinguishing sorbitol from other polyols, although a trace of a compound with a rate of movement on the chromatogram less than that of sorbitol, and corresponding to myoinositol, was detected. Further evidence that little, if any, acyclic polyol other than sorbitol is present in plum leaves in the free state was obtained by the agreement between an estimate of the concentration of acyclic polyol in a non-radioactive plum-leaf

extract by the adapted periodate method (Found: 0.67 g./l.) and an isotopic-dilution assay for sorbitol in the same extract (Found: 0.68 g./l.), with [^{14}C]sorbitol purified via its crystalline hexaacetate as carrier.

As shown in Table 1, the yield of crude sorbitol from the leaves of each photosynthesis experiment was approximately the same, relative to the quantity of leaves used, and corresponded to 38–45 mg./g. dry wt. of leaf. No systematic variation in these values was apparent, but judging from the weights of D-glucose isolated from hydrolysates of the polysaccharide fractions of the same leaves, considerable net synthesis of starch occurred during the same period.

The quantity of impure sorbitol isolated from the leaves, before chromatographic purification, was considered a good measure of the amount initially present, but experience indicated that the losses incurred in isolating and purifying the glucose were 40–60%, depending on the amount involved. Therefore in calculating the approximate incorporation of ^{14}C into sorbitol and starch, allowance was made for a 50% loss of glucose in the preparation, and the resulting uncertainty in

the glucose figure is indicated in Table 2, where the results are depicted together with other figures relating to ^{14}C distribution in the leaves. After photosynthesis for 6 hr. practically all of the $^{14}\text{CO}_2$ supplied to the leaves had been metabolized (Andrews & Hough, 1958) and, in these circumstances, since the same amount of carbon dioxide was supplied each time, the resultant specific activities of the leaf components would be related inversely to the weights of the leaves used. Accordingly the specific activities of sorbitol at 6, 15.5 and 24 hr. were corrected to a common basis of 3 g. of alcohol-insoluble leaf residue per sample (actual weights were 3.12, 3.72 and 2.97 g. respectively). In every experiment a large proportion of the ^{14}C in alcohol-soluble compounds was present in the sorbitol, demonstrating a rapid and high degree of incorporation of isotope into this polyol. In each case there was also considerably more ^{14}C in sorbitol than in starch. The results in Table 1 indicate that no net synthesis of sorbitol occurred during photosynthesis, yet the specific activity of ^{14}C in sorbitol relative to that of the $^{14}\text{CO}_2$ supplied (Table 2) shows that, after photosynthesis for only 3 hr. in a high $^{14}\text{CO}_2$ concentration, 15% of the sorbitol was newly synthesized from labelled precursors. This corresponds to the synthesis and utilization of about 2.0 mg. of sorbitol by each leaf. Clearly there was a rapid turnover of the sorbitol pool in equilibrium with the primary products of photosynthesis. The specific activity of ^{14}C in starch was different in each polysaccharide fraction examined (P. Andrews & L. Hough, unpublished work), but usually about twice that of ^{14}C in sorbitol. After photosynthesis for 3 hr. roughly 0.7 mg. of starch had been synthesized per leaf from labelled precursors.

The apparent invariance of sorbitol concentration in plum leaves under the rather extreme conditions of the photosynthesis experiments was unexpected, but seemed worthy of further investigation, particularly with leaves still growing on the tree. The method for estimating sorbitol in bio-

Table 1. *Glucose and sorbitol isolated from photosynthesizing plum leaves*

For experimental details see text.

Period of photosynthesis (hr.)	Yield corresponding to 3 g. of alcohol-insoluble residue*	
	Sorbitol (mg.)	D-Glucose† (mg.)
1.5	275	†
3	330	18
6	270	25
15.5	310	29
24	280	53

* In separate experiments the actual weight of alcohol-insoluble residue was in the range 2.5–3.7 g.

† From polysaccharide hydrolysates.

‡ Insufficient glucose was present to permit its isolation and purification.

Table 2. *Distribution of ^{14}C in plum-leaf constituents after photosynthesis in $^{14}\text{CO}_2$*

See text for experimental details.

Period of photosynthesis (hr.)		1.5	3	6	15.5	24
As % of ^{14}C supplied	Total ^{14}C in leaves	34	79	98	97	91
	^{14}C in alcohol-soluble fraction	25	57	59	53	52
	^{14}C in sorbitol	7	17	21	30	22
	^{14}C in starch	—	5±2	8±2	11±2	17±3
As % of total ^{14}C in leaves	^{14}C in alcohol-soluble fraction	72	68	59	58	57
	^{14}C in sorbitol	20	22	22	31	25
	^{14}C in starch	—	6±2	8±2	11±2	19±3
	^{14}C in sorbitol (as % of ^{14}C in alcohol-soluble materials)	28	32	39	53	43
Specific activity of ^{14}C in sorbitol		0.08	0.16	0.23	0.26	0.22
Specific activity of $^{14}\text{CO}_2$ supplied						

logical materials described by Adcock (1957) gave inconsistent results with plum-leaf extracts. The improvement suggested by White (1958) to remove interfering non-reducing carbohydrates may well have been effective here, but a simplified method with ion-exchange resins to hydrolyse, degrade and remove interfering substances was devised instead.

Sucrose is an alcohol-soluble constituent of plum leaves, and White (1958) showed that it interferes with Adcock's method of sorbitol estimation. However, the simplified method gave satisfactory results with mixtures of sorbitol and sucrose,

Table 3. *Estimation of sorbitol admixed with sucrose*

Figures are concentrations in g./l.

Composition of mixture		Sorbitol found
Sucrose	Sorbitol	
0.484	0.400	0.416
0.646	0.788	0.780
0.568	2.232	2.280

Table 4. *Estimation of sorbitol added to plum-leaf extracts*

Figures are concentrations in g./l.

Sorbitol added	Estimated increment in sorbitol
(A) With an extract of leaves picked at 3 a.m. Initial sorbitol concentration: 0.566 g./l.	
0.368	0.372
0.734	0.730
1.218	1.140
2.042	1.815
(B) With an extract of leaves picked at 8 p.m. Initial sorbitol concentration: 0.604 g./l.	
0.276	0.278
1.804	1.750

Table 5. *Sorbitol content of plum leaves during 21 hr. under natural conditions*

Fifteen leaves were picked at each time, giving 2-3 g. of alcohol-insoluble leaf residue.

Time of sampling	Sorbitol in alcohol extract (mg./g. of alcohol-insoluble leaf residue)
1 p.m.	106
5 p.m.	112
8 p.m.	111
Midnight	112
3 a.m.	110
5 a.m.	105
9 a.m.	109

initially dissolved in 95 % ethanol to simulate leaf extracts and containing 0.6-4 times the concentration of sorbitol found in plum-leaf extracts (Table 3). To test for possible diurnal variations in substances interfering with estimations by the periodate method, the sorbitol concentrations in extracts of plum leaves picked at 3 a.m. and 8 p.m. were determined before and after the addition of known amounts of sorbitol. The results (Table 4) again were satisfactory.

The method was then applied to the estimation of sorbitol in extracts of plum leaves picked from three trees at intervals during a 20 hr. period, commencing at 1 p.m. one day and ending at 9 a.m. the next day. The results (Table 5) for each sample do not differ significantly from their mean value of about 110 mg. of sorbitol/g. of alcohol-insoluble leaf residue (corresponding to about 45 mg./g. dry wt. of leaf), although the leaves were in darkness from about 10 p.m. to 4 a.m. Paper-chromatographic examination of the extracts indicated that sorbitol was the only polyol constituent, except for traces of *myoinositol*, which does not give formaldehyde on periodate oxidation. The evidence from this experiment and from the photosynthesis experiments with $^{14}\text{CO}_2$ indicates that the total free sorbitol content of plum leaves is independent of the photosynthetic activity of the leaves, both under natural conditions and during a period of very rapid sorbitol synthesis after a prolonged spell in darkness.

These results may be compared with findings of Hutchinson, Taper & Towers (1959), who observed a high degree of incorporation of $^{14}\text{CO}_2$ into the sorbitol of apple leaves and suggested that the polyol has a central role in metabolism of the leaves. A large incorporation of $^{14}\text{CO}_2$ into the D-mannitol of *Fucus vesiculosus* has likewise been reported (Bidwell, Cragie & Krotkov, 1958). A metabolic function of a polyol which has been conclusively demonstrated is the interconversion of *threo*-D- and -L-pentulose (xylulose) via xylitol in guinea-pig liver (Hollman & Touster, 1957), and so it seems possible by analogy that sorbitol is an intermediate in the interconversion of D-glucose, D-fructose and L-sorbose.

SUMMARY

1. Plum leaves, which are rich in sorbitol, utilized a higher proportion of the ^{14}C incorporated during photosynthesis in $^{14}\text{CO}_2$ for sorbitol synthesis than for starch synthesis.

2. Despite the large amounts present in the leaves, sorbitol is rapidly equilibrated with the primary products of photosynthesis; 15 % was newly synthesized from ^{14}C -labelled precursors in 3 hr.

3. The method of Adcock (1957) for estimating sorbitol in biological material was adapted for use with leaf extracts.

4. The sorbitol content of plum leaves under natural conditions was followed from 1 p.m. on one day to 9 a.m. the next day. The amount present remained constant at 110 mg./g. of alcohol-insoluble leaf residue (45 mg./g. dry wt. of leaf).

The investigation formed part of the programme of the Food Investigation Organisation of the Department of Scientific and Industrial Research. The authors wish to thank Mr E. W. Hobbs and the Director of the Long Ashton Agricultural and Research Station for facilities placed at their disposal, and Dr D. I. Rees for helpful advice.

REFERENCES

- Adcock, L. H. (1957). *Analyst*, **82**, 427.
 Altermatt, H. A. & Neish, A. C. (1956). *Canad. J. Biochem. Physiol.* **34**, 405.
 Anderson, J. D., Andrews, P. & Hough, L. (1959). *Biochem. J.* **72**, 9p.
 Andrews, P. & Hough, L. (1958). *J. chem. Soc.* p. 4483.
 Barker, S. A. (1955). In *Modern Methods of Plant Analysis*, p. 55. Ed. by Paech, K. & Tracey, M. V. Berlin: Springer Verlag.
 Bidwell, R. G. S., Cragie, J. S. & Krotkov, G. (1958). *Science*, **128**, 776.
 Donen, I. (1939). *Biochem. J.* **33**, 1611.
 Hollman, S. & Touster, O. (1957). *J. biol. Chem.* **225**, 87.
 Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.
 Hutchinson, A., Taper, C. D. & Towers, G. H. N. (1959). *Canad. J. Biochem. Physiol.* **37**, 901.
 Kidd, F., West, C., Griffiths, D. G. & Potter, N. A. (1938). *D.S.I.R. Fd Inv. Board Report*, p. 132.
 O'Dea, J. F. & Gibbons, R. A. (1953). *Biochem. J.* **55**, 580.
 Rees, W. R. & Reynolds, T. (1958). *Nature, Lond.*, **181**, 767.
 Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.
 Verley, A. & Bölsing, F. (1901). *Ber. dtsh. chem. Ges.* **34**, 3354.
 White, J. W. (1958). *Analyst*, **83**, 649.

Biochem. J. (1961) **81**, 154

A New Asparagine Derivative, N⁴-(2-Hydroxyethyl)-L-asparagine, from Bryony (*Bryonia dioica*)

By L. FOWDEN

Department of Botany, University College London, Gower Street, London, W.C. 1

(Received 15 March 1961)

Many amino acids additional to those normally present in protein have been identified as components of higher plants (see reviews, Hunt, 1959; Fowden, 1958, 1960). Several of the newly characterized acids are derivatives of either glutamic acid or glutamine; others based on aspartic acid or asparagine are now known. N⁴-Ethyl-L-asparagine (I) was isolated recently from squirting cucumber (*Ecballium elaterium*) by Gray & Fowden (1961). The present paper reports the isolation of larger quantities from another member of the Cucurbitaceae, *Bryonia dioica* (white bryony), together with another substituted amide identified as N⁴-(2-hydroxyethyl)-L-asparagine (II).

ISOLATION AND IDENTIFICATION

The substances were encountered as ninhydrin-reacting spots (brown) on paper chromatograms of 75% (v/v) ethanol extracts of macerated bryony

shoots. About 7 g. of ethylasparagine and 1.2 g. of hydroxyethylasparagine were isolated from an aqueous extract of 20 kg. of shoots. Hydroxyethylasparagine, having empirical formula C₈H₁₂O₄N₂, gave equimolar amounts of aspartic acid and ethanolamine after acid hydrolysis. Extracts of rat liver also converted hydroxyethylasparagine into aspartic acid and ethanolamine; ethylasparagine was converted similarly into aspartic acid and ethylamine. The pK_a values of hydroxyethylasparagine were 2.32 and 8.92 [cf. values for asparagine, 2.02 and 8.80 (Meister, 1957)]. Ammonia displaced hydroxyethylasparagine from cation-exchange resin columns together with glutamic acid and before displacement of ethylasparagine and asparagine. This result was in agreement with structure for hydroxyethylasparagine which contains a free hydroxyl group; a possible alternative structure, 4-(2-aminoethyl)-hydrogen aspartate, an ester of ethanolamine and aspartic acid, presumably would behave as a basic amino acid on ion-exchange resins.

Confirmation of the hydroxyethylasparagine structure was obtained by chemical synthesis. The