The Partial Purification of Parathyroid Hormone by Means of Ultrafiltration and Displacement Chromatography

By H. RASMUSSEN* AND R. G. WESTALL

Medical Unit, University College Hospital Medical School, London, W.C. 1

(Received 23 April 1957)

Although apparently the first effective parathyroid extract was prepared by Moussu in 1898, some time elapsed before Hanson (1925) and Collip (1925) clearly demonstrated that an active extract of consistent potency could be prepared by using hot dilute hydrochloric acid. Since that time there have been a number of attempts to purify such extracts further. Some further purification was achieved by Ross & Wood (1942) and L'Heureux, Tepperman & Wilhelmi (1947) by means of ammonium sulphate fractionation and fractional precipitation with acetone respectively. Several brief reports, including the one of Handler, Cohn & Dratz (1954), have appeared on the unsuccessful attempts to use chromatography for isolation of the active principle.

Because they felt that initial extraction with hydrochloric acid might be disadvantageous in later purification procedures, Davies & Gordon (1955) adopted acetic acid as the extracting agent. They showed that the active principle could be obtained by extraction with 80% acetic acid, and further demonstrated that 50–65% of the extracted hormonal activity would pass a cellophan membrane during ultrafiltration at pH 2.4. Their most potent preparations had a specific activity comparable with, or slightly greater than, those of L'Heureux *et al.* (1947).

The studies reported in this communication are concerned with an evaluation of these two acid extraction procedures and the further purification of the extracts by fractional precipitation with acetone, ultrafiltration, and displacement chromatography from ion-exchange resins. By these means a hormonally active material with a specific activity of 75–100 units/mg. dry weight has been prepared. In addition, evidence is presented which suggests that the active material obtained by means of acetic acid and hydrochloric acid extraction respectively have different chemical properties.

EXPERIMENTAL

Assay method

The increase in the concentration of calcium in the plasma of parathyroidectomized rats, 6–18 hr. after the test injection, was used as the basis of all assays. Initially an 18 hr. assay

was used. This was similar to that described by Davies, Gordon & Mussett (1954), except that smaller rats (120–130 g.) were employed, and the concentration only of plasma calcium, rather than of both calcium and magnesium, was measured. Also the animals were initially used 4 days after parathyroidectomy and every 4 days thereafter for a maximum of four times and then discarded. The smaller animals were more responsive to a standard dose of Parathormone (Eli Lilly and Co.), 50 units eliciting an increase in plasma calcium of 2.2 mg./100 ml. 18 hr. after injection, compared with an increase of 1.8 mg./100 ml. after 60 units in the larger animals used by Davies *et al.* (1954). A straight-line relationship was found between the increase in plasma calcium concentration and log dose of hormone over the range of 25–125 units. Those animals whose initial calcium concentrations were 8.5 mg./100 ml. or more were not used, so that the initial plasma-calcium concentrations ranged between 6.5 and 8.4, with a mean of 7.3 mg./100 ml. Within this range it was not possible to establish any dependence of the response on the initial calcium concentration as described by Davies *et al.* (1954), even though this was evaluated in all assays performed over a 10 month period.

For screening a large number of fractions for hormonal activity 6–8 rats/fraction were used and cross-over experiments were not carried out. In this way approximate potencies were determined. When a more precise measurement of potency was desired a cross-over design similar to that of Davies *et al.* (1954) was employed. A group of 32 animals was divided into four groups; two groups received 50 and 100 units respectively of the standard (Parathormone, Eli Lilly) and the others received two different doses of the unknown, calculated as nearly as possible to contain 50 and 100 units of activity. The limits of error of this method were 75–130% when expressed as a percentage of potency.

As the purification studies progressed it was found that the time-course of action of the active material was altered (Rasmussen & Westall, 1956). The maximum response occurred at 6 rather than 18 hr. and the effect was over in 14–16 hr.

This assay method could be used, however, if the material to be tested was suspended in arachis oil rather than dissolved in 0.9% saline before injection. When the partially purified extracts were suspended in oil their time-course of action was similar to that of the crude extracts. Suspension of the crude material in oil did not change its time-course of action.

It was found more convenient to use a shorter assay. A method similar in detail to the one above was developed, except that the second bleeding was 6 rather than 18 hr. after injection. The materials to be tested were all dissolved in 0.9% saline before injection, and both the crude and more purified material could be assayed. The limits of error were found to be similar to those above. Although the maximum

* Present address: Rockefeller Institute for Medical Research, New York City.

response to the standard was 15–20 hr., the response at 6 hr. to 50 units was only slightly less (1.9 compared with 2.2 mg./100 ml.) and was no more variable. When the same partially purified preparation was tested by the 6 hr. (saline) and 18 hr. (oil) methods, no difference in potency was found.

Plasma-calcium estimations

Blood (0.8–1.0 ml.) was drawn into a heparinized syringe by cardiac puncture under light ether anaesthesia. The plasma was separated as soon as possible. Calcium was determined on 0.2 ml. samples of plasma by titration at pH 12 with sodium ethylenediaminetetra-acetate (EDTA) in a Unicam SP. 600 spectrophotometer at 510 m μ , with ammonium purpurate as an indicator. The percentage transmission was recorded on semi-log graph paper after the addition of each measured increment of standard EDTA. When these readings were plotted the resulting line showed two slopes, the intersection of which was taken as the end point. This method was reported by Kenny & Toverud (1954).

Preparation of fat-free gland powder

Fresh, frozen, bovine glands were minced in a meat grinder by using a plate with $\frac{1}{8}$ in. holes. The ground tissue was placed immediately in cold acetone (0°) and reminced with a plate having $\frac{1}{16}$ in. holes. It was then extracted with acetone and chloroform as described by L'Heureux *et al.* (1947). The fresh glands (1 kg.) yielded approximately 100 g. of dry powder. The dry powder was stored in a glass-stoppered bottle at 0°, and was stable for at least 4 months. This powder was the starting material for all subsequent work.

Preparation of acetic acid extract

The procedure of Davies & Gordon (1955) was used, with some modifications. The defatted powder was dissolved in 80% (v/v) acetic acid (12 ml./g. of dry powder), heated with stirring to 75° and kept at this temperature for 5 min. The mixture was then cooled to 40°, filtered through six layers of cheese-cloth, re-extracted for 5 min. with another volume of 80% acetic acid at 75° and refiltered. The combined filtrates were cooled to 10° and 3 vol. of cold acetone (0°) was added. This mixture was allowed to stand for 1 hr. at 0°. The precipitate which formed was removed by centrifuging and discarded. Acetone was then added to the supernatant fluid to a final concentration of 90%, and the mixture allowed to stand 2–3 hr. at 0°. A precipitate formed which settled to the bottom of the flask during this time. The bulk of the clear supernatant fluid was removed by decantation and the precipitate then collected by centrifuging, washed four times with acetone and dried *in vacuo* at 0° over H₂SO₄. From 60 g. of defatted powder, approximately 3 g. of a pale-brown powder was obtained.

Preparation of hydrochloric acid extract

The procedure of L'Heureux *et al.* (1947) was used, except that the final precipitation with 90% acetone at neutrality was omitted owing to difficulties in controlling the pH. In the method used the powder was extracted with 0.2N-HCl (10 ml./g. of powder) at 75° for 5 min. The mixture was cooled to 45° and centrifuged, and the residue re-extracted with another volume of 0.2N-HCl at 75° for 5 min. The combined supernatant fluids were cooled to 10°. Acetone (4 vol.) was then added, and the mixture was allowed to

stand 1 hr. at 0° to ensure maximal precipitation and then centrifuged to remove the precipitate. The supernatant fluid was freed from acetone by distillation under reduced pressure at a temperature not exceeding 40°. The resulting acid solution (pH 1.8) was then ultrafiltered.

Ultrafiltration

Ultrafiltration was carried out by using sacs of Visking dialysis tubing ($1\frac{1}{8}$ in. in diameter, 15 cm. long) which were reinforced with an outer sheath of open-mesh silk fabric. The construction of the sacs was demonstrated to us by Dr A. H. Gordon. Thus supported they gave adequately rapid filtration (5 ml./hr.) and could withstand a pressure of 720 mm. Hg.

For ultrafiltration, 1 g. of the powder obtained by acetone precipitation of the acetic acid extract was dissolved in 100 ml. of either 2.2N-acetic acid (pH 2.35) or 0.2N-formic acid (pH 2.05).

Ion-exchange columns

Both Dowex 50 (2% cross-linked) and Dowex 50 (4% cross-linked) have been used in our studies. A column containing a bed of 200–400 mesh resin 20 cm. deep and 5.5 cm. in diameter served for the fractionation of the ultrafiltrate obtained from 60 g. of the acetone-dried powder. The resin, which was used in the H⁺ ion form, was allowed to stand for 1 hr. in 2N-HCl, washed twice with water by decantation and then poured into the column as a slurry. Before use, the packed column was washed with distilled water until the pH of the effluent was almost neutral. A smaller column of the anion-exchange resin Dowex 2 (8% cross-linked) was also used. The bed of 200–400 mesh resin was 10 cm. deep and 1.5 cm. in diameter. This resin was used, according to need, either in the OH⁻ ion form, obtained by treatment with N-NaOH and then distilled water, or in the formate form, achieved by saturating the resin with 1.0N-formic acid before packing and washing the column.

RESULTS

Initial extraction

The dilute hydrochloric acid and the acetic acid methods were about equally effective in extracting hormonal activity from the acetone-dried powder. A weight of 60 g. of powder yielded 10 000–12 000 U.S.P. units.

Acetone fractionation

Initially the procedure of L'Heureux *et al.* (1947) was employed to obtain an active powder from the crude hydrochloric acid extract. The crucial step in their procedure was the neutralization of the acid acetone solution, followed by the addition of more acetone to a final concentration of 90%. We were able to obtain an active powder by this means which had a potency of 20–25 units/mg. dry weight. However, great difficulty was encountered in controlling the pH during neutralization, since the protein which precipitated seemed to carry the water with it and selectively concentrate the sodium hydroxide so that the pH of the protein solution in the bottom of the flask was strongly

alkaline at a time when the supernatant was still acid. In addition, when the supernatant from the final acetone precipitation was concentrated and assayed, it was found to contain approximately 10% of the estimated original activity. Because of these two reasons this procedure was abandoned.

Ultrafiltration

When the dilute hydrochloric acid extracts were ultrafiltered, under optimum conditions 90–95% of the hormonal activity passed through the membrane. This value, however, was not always attained. The yield was found to be correlated with the fat content of the original dried powder, completely fat-free powders giving the highest yield. In one instance when poorly defatted powder was used the yield was approximately 50%. When the residue in the sac was lyophilized, re-extracted with chloroform and acetone, dissolved in 0.2N-hydrochloric acid and again subjected to ultrafiltration, 90% of the estimated activity then passed through the membrane.

Ultrafiltration of the 80% acetic acid extract confirmed the finding of Davies & Gordon (1955) that 60–65% of the hormonal activity passed through the cellophan membrane. Their studies were based primarily on a mouse-urine phosphate-assay method (Davies, Gordon & Mussett, 1955), but they also assessed blood-calcium-raising activity and reported a value of approximately 20 units/mg. dry wt. A similar activity was found with our material.

With the acetic acid extracts we were never able to get more than a 65% yield in the ultrafiltrate, even from well defatted powders when 2.2M-acetic acid was used. There is no doubt that the acetic acid extracts had a larger percentage of inert material since it had a lower specific activity and was much more viscous than the hydrochloric acid extracts. It was possible to increase the yield, however, by dissolving the protein material in 0.2N-formic acid rather than in 2.2N-acetic acid. Under these conditions the fluid appeared to be less viscous and 80–90% of the activity passed through the membrane, with a corresponding increase in specific activity of the ultrafiltrate (Table 1).

Displacement chromatography of hydrochloric acid extract

In a typical experiment the extraction of 60 g. of dried powder with 0.2N-hydrochloric acid yielded 600–700 ml. of ultrafiltrate. This volume was doubled by the addition of water to reduce the acid concentration. This solution was allowed to run on to a Dowex 50 resin column at a rate not exceeding 3 ml./min. A definite, but only faintly visible, band was seen to form and to progress slowly down the column. The front of this band extended to a point

about two-thirds of the way down the column when all the diluted ultrafiltrate had been applied. The column was then washed with 0.01N-hydrochloric acid until the effluent no longer absorbed light at 225 m μ (measured in a Unicam SP. 500 spectrophotometer).

Displacement of the substance held by the resin was then carried out at a flow rate of about 30 ml./hr. with a solution obtained by mixing equal volumes of 0.2N-sodium hydroxide and 0.02N-hydrochloric acid. As displacement proceeded a band of white insoluble material (later tentatively identified as guanine) formed just ahead of the sodium front (dark band). This precipitated material tended to distort the sodium front, but did not interfere unduly with development provided that the recommended flow rate was not exceeded. The faint protein band did not move forward until the sodium front approached within 6–8 cm. of it.

Collection of the effluent in 10–20 ml. fractions began as soon as increased absorption at 225 m μ was observed, and continued until there was a sharp rise in absorption at 260 m μ indicating breakthrough of the purine front (Fig. 1). The fractions which contained protein, but not purine, were pooled and passed through a column of Dowex 2 resin in the formate form and then lyophilized. The exchange of formic for hydrochloric acid permitted a more

Fig. 1. Ultraviolet absorption at 225 m μ (●) and 260 m μ (○) of fractions obtained by displacement chromatography of parathyroid hormone on Dowex 50 (4% cross-linked) (200–400 mesh). The material applied to the column was an ultrafiltrate of hydrochloric acid extract of dried glands. The developing solution was 0.09M-NaOH–0.01M-NaCl. Fractions were 15 ml. each, and the hormonal activity was found in fractions 6–25. The sharp rise in absorption at 260 m μ beginning in fraction 26 indicates the breakthrough of the purine band.

satisfactory lyophilization. The powder from the lyophilized fractions was found to have an activity of from 75 to 100 units/mg. dry wt. Some further hormonal activity was always found in the effluent obtained during the development of the purine band due to the previously mentioned irregularity in this front. This material could be recovered by passing it through a Dowex 2 column in the OH^- ion form to remove the purine, reacidifying and re-running it on a smaller Dowex 50 column.

From 60 g. of the original fat-free gland powder 90–100 mg. of this highly active material was obtained. It has been stored for as long as 6 months, as a lyophilized powder in a vacuum desiccator at room temperature, without change in potency.

Displacement chromatography of acetic acid extracts

The powder obtained by lyophilization of the ultrafiltrate of the acetic acid extract (obtained from the same fat-free gland powder as the hydrochloric acid extract) was taken up in 0.1N-hydrochloric acid and applied to a Dowex 50 (4% cross-linked) column in the H^+ ion form. However, with these extracts no visible band formed on the column and it was established that the active material was not retained by the resin but passed straight through the column, so that purification was not achieved. Heating this powder to 75° for 5 min. in 0.2N-hydrochloric acid did not change its chromatographic behaviour. Likewise, heating the hydrochloric acid ultrafiltrates in 80% acetic acid for 5 min. at 75° did not alter its chromatographic behaviour. This would seem to indicate a difference between the active molecules obtained by means of the two acid-extraction procedures, and an inability to convert one into the other, at least in the circumstances described.

Specific activity

Table 1 summarizes the data on the degree of purification achieved by each of the procedures employed, as well as the average recovery of

originally extracted activity. The potency of the original acetic acid extract was approximately one-half that of the hydrochloric acid extract. Both were purified to approximately the same degree by acetone fractionation and ultrafiltration, but only the hydrochloric acid extract was further purified by displacement chromatography on Dowex 50 resins. This highly active material (75–100 units/mg. dry wt.) had a nitrogen content of 14.0% (micro-Kjeldahl), which indicated a specific activity of 500–700 units/mg. of N.

When the active precipitate obtained by acetone fractionation of the acetic acid extract was dissolved in 0.2N-formic acid (Table 1b) rather than 2.2N-acetic acid (Table 1a) a greater percentage of the activity passed through the cellophan and the specific activity of the ultrafiltrate was correspondingly higher (30–35 rather than 15–20 units/mg. dry wt.) and the overall recovery was consequently greater (80–90% as compared with 50–60%).

Electrophoresis and ultracentrifugation

Preliminary studies have been carried out on our most potent hydrochloric acid-extracted preparation (100 units/mg. dry wt.). Electrophoresis in a Tiselius apparatus was performed by K. Schmid (Massachusetts General Hospital, Boston, Mass.), using a glycine buffer, pH 2.5. The pattern obtained showed that the material was heterogeneous, consisting of two and possible three components. Ultracentrifuging performed by A. H. Gordon and P. A. Charlwood (National Institute for Medical Research, London, N.W. 7) showed that the material behaved as if it consisted primarily of a single component with an estimated molecular weight of 4000–6000.

Other constituents of parathyroid glands

During the course of this work, we have gained some knowledge about the chemical constituents of parathyroid-gland tissue. Apart from the tissue

Table 1. *Purification of parathyroid hormone*

Specific activities after each purification procedure, and the percentage of the activity in the original extracts recovered after each step are given for material extracted initially with either 80% acetic acid or 0.2N-HCl (see text). In the 80% acetic acid columns (a) and (b) refer to the specific activities and yields obtained with 2.2N-acetic and 0.2N-formic acid respectively, during the ultrafiltration step.

Stage of preparation	Hormonal activity (units/mg. dry wt.)		Recovery (%)	
	80% acetic acid	0.2N-HCl	80% acetic acid	0.2N-HCl
Original extract	3–5	8–10	100	100
Supernatant from 75% acid, acetone fractionation	—	12–15	—	100
Precipitate from 90% acetone fractionation	7–10	20–25	90–95	80
Ultrafiltrate	(a) 15–20 (b) 30–35	30–40	(a) 50–60 (b) 80–90	90–95
Effluent after displacement chromatography on Dowex 50 (4% cross-linked)	—	75–100	—	75–80

protein which is removed by acetone precipitation and ultrafiltration there is a considerable amount of purine tentatively identified as guanine by means of paper chromatography, paper electrophoresis, and a positive Pauly reaction. The effluent from the Dowex 50 column contains at least two recognizable substances: one has been identified as inositol, m.p. 224° [mixed m.p. with mesoinositol (m.p. 225°), 224°] (Found: C, 39.7; H, 6.5. Calc. for $C_6H_{12}O_6$: C, 40.0; H, 6.7 %). The other is a peptide (shown by liberation of amino acids on acid hydrolysis) which is held by and can be displaced from a Dowex 2 column. In addition there are free amino acids.

DISCUSSION

Because the initial electrophoresis experiments showed that the most active material was heterogeneous, further characterization was not done. From these data and the results of the ultracentrifuge experiment, it would appear that our most active preparation, obtained from the hydrochloric acid extract by means of ultrafiltration and displacement chromatography on Dowex 50 resin, is a mixture of polypeptides of approximately the same size, in a molecular-weight range of 4000–6000. This is similar to the findings of Davies & Gordon (1955) with acetic acid extracts of lower hormonal potency. Our active material has a potency of 500–700 units/mg. of nitrogen. This is approximately twice the potency found by L'Heureux *et al.* (1947) in material prepared by acetone fractionation. The basis of their assay method was the fall in serum inorganic phosphorus concentration of normal rats (size not stated) 3 hr. after the injection of the test material (Tepperman, L'Heureux & Wilhelmi, 1947). Because of the difference in assay method it is difficult to make a strict comparison. However, an extract prepared according to their method was found to have an activity of approximately 25 units/mg. dry wt. by our assay method. The material we obtained by displacement chromatography is the most active yet reported, except for that described by Raoul, Marnay & Prelot (1955). Upon repeating their work with a more reliable assay method it has not been possible to confirm their findings (Rasmussen & Westall, 1957). Actually, as reported, no hormonal activity could be demonstrated in their so-called 'lipoid fractions', and 80 % of the estimated original activity was found in the protein residues, results completely contradictory to theirs. Because Raoul *et al.* (1955) used rabbits as assay animals it is difficult to accept their findings, particularly since Dyer (1935), who developed the method, concluded that it was unsuitable for quantitative assays. Certainly the majority of past evidence [summarized by Greep (1948) and Greep & Kenny (1955)], as well as the recent work of

Davies & Gordon (1955) and our own experience, indicate that this hormone is a protein.

Our experience with ultrafiltration confirms and extends the work of Davies & Gordon (1955). To obtain maximal yield during the procedure, it is necessary that the initial fat extraction be thorough. This does not wholly explain the difference in yield obtained with the two different acid extracts. The greater viscosity of the acetic acid extracts may, in part, explain the lower yield. The improvement of yield with 0.2N-formic acid suggests that pH may be important in controlling the degree of dissociation between the active hormone and larger proteins. This might also explain why high yields are obtained with hydrochloric acid extracts. Alternatively, it may be that the hormone exists as a larger complex in the natural state and is more thoroughly cleaved into smaller, but still active, units by the hydrochloric acid.

Although by using the chromatographic conditions described we were able to carry out successful displacement development, the performance was not ideal. This was due primarily to the precipitation of purine in front of the sodium band which interferes with displacement development. As pointed out, this difficulty was minimized if the recommended flow rate was not exceeded. Apparently the sequence of events on the column is as follows: the purine is soluble in the acid solution in which it is applied to the column; when displacement development is begun, the sodium in the developing solution displaces the purine from the column, but because the conditions at this point are close to neutrality the purine precipitates; then as the resin is saturated with sodium the pH rapidly rises to 12–13 and the purine redissolves. So far we have been unable to circumvent this difficulty, but it may be possible to separate the purine from the protein before displacement chromatography by use of a dialysis cell similar to that described by Craig & King (1955). The use of dilute chloride (0.01N) in the developing solution was necessary to provide acidic conditions in the ambient solution ahead of the sodium front in order to prevent precipitation of protein on the resin. If the sodium hydroxide was replaced by either ammonium hydroxide (0.1N) or glycine (0.1N) in the developing solution, displacement of the protein band did not occur; instead the protein remained on the resin and was overrun by the base. Because the rate of equilibration of the protein with the resin is quite slow it is imperative to apply the protein solution slowly. Even with the rate of application recommended it is obvious that complete saturation of the resin did not occur, since the protein front did not begin to move until the sodium front had progressed approximately one-third the way down the column. The reason for part of this behaviour is no doubt that the purine which

precipitates no longer takes up its space on the resin.

Because of the difference in behaviour of the acetic acid and hydrochloric acid extracts when subjected to the same chromatographic procedure it seems safe to conclude that they differ chemically. This is further borne out by the difference in their stability in various buffer systems, and their electrophoretic properties (Dr H. Rasmussen, unpublished work). For sake of reference, we have called the active material extracted by hydrochloric acid, Parathormone A (PTH-A) and that extracted by acetic acid, Parathormone B (PTH-B).

SUMMARY

1. A method is described for the partial purification of parathyroid hormone by means of hydrochloric acid extraction, acetone fractionation, ultrafiltration and displacement chromatography on ion-exchange resin.

2. The procedure yields material of potency 75–100 units/mg. dry weight (500–700 units/mg. of N) in quantities of 90–100 mg./600 g. of fresh bovine glands.

3. This material is heterogeneous on electrophoresis at pH 2.5.

4. Evidence is presented which suggests that the active material obtained by means of acetic acid and hydrochloric acid extraction respectively have different chemical properties.

We wish to thank Dr A. H. Gordon for his constant interest and many helpful suggestions. Our thanks also to Professor C. E. Dent and Professor M. Rosenheim for their support of, and interest in, this work, which was initiated at their suggestion. This work was supported in part by grant A-1331 of the U.S. Public Health Service, and was carried

out while one of us (H.R.) was a recipient of a U.S. Public Health Research Fellowship.

REFERENCES

- Collip, J. B. (1925). *J. biol. Chem.* **63**, 395.
 Craig, L. C. & King, T. P. (1955). *J. Amer. chem. Soc.* **77**, 6620.
 Davies, B. M. A. & Gordon, A. H. (1955). *Biochem. J.* **61**, 646.
 Davies, B. M. A., Gordon, A. H. & Mussett, M. V. (1954). *J. Physiol.* **125**, 383.
 Davies, B. M. A., Gordon, A. H. & Mussett, M. V. (1955). *J. Physiol.* **130**, 79.
 Dyer, F. J. (1935). *Quart. J. Pharm.* **8**, 197.
 Greep, R. O. (1948). *Physiology and Chemistry of Parathyroid Hormone*. In *The Hormones*, vol. 1, ed. by Pincus, G. & Thimann, K. V. New York: Academic Press Inc.
 Greep, R. O. & Kenny, A. D. (1955). *Physiology and Chemistry of the Parathyroids*. In *The Hormones*, vol. 3, ed. by Pincus, G. & Thimann, K. V. New York: Academic Press Inc.
 Handler, P., Cohn, D. V. & Dratz, A. F. (1954). *Studies on the Purification of Parathyroid Extract*. In *Metabolic Interrelations with Special Reference to Calcium*. Trans. 5th Josiah Macy Conf. p. 320. New York: Progress Associated Inc.
 Hanson, A. M. (1925). *Proc. Soc. exp. Biol., N.Y.*, **22**, 560.
 Kenny, A. D. & Toverud, S. U. (1954). *Analyt. Chem.* **28**, 1059.
 L'Heureux, M. V., Tepperman, H. M. & Wilhelmi, A. E. (1947). *J. biol. Chem.* **168**, 167.
 Moussu, G. (1898). *C.R. Soc. Biol., Paris*, **5**, 867.
 Raoul, M. Y., Marnay, C. & Prelot, M. (1955). *C.R. Acad. Sci., Paris*, **240**, 1151.
 Rasmussen, H. & Westall, R. G. (1956). *Nature, Lond.*, **178**, 1173.
 Rasmussen, H. & Westall, R. G. (1957). *Nature, Lond.* (in the Press).
 Ross, W. F. & Wood, T. R. (1942). *J. biol. Chem.* **146**, 49.
 Tepperman, H. M., L'Heureux, M. V. & Wilhelmi, A. E. (1947). *J. biol. Chem.* **168**, 151.

The Structure of Serratamic Acid

By N. J. CARTWRIGHT

Department of Bacteriology, University of Birmingham

(Received 22 May 1957)

In a previous communication (Cartwright, 1955) the isolation of serratamic acid, a metabolic product of organisms of the *Serratia* group, was described. On the basis of the nature of its hydrolytic products and lack of ninhydrin reaction a peptide structure was advanced and this has now been confirmed by synthesis. In particular, L-serine was rigidly established as a product of acidic hydrolysis. Both alkaline and acidic hydrolysis yielded an acid,

$C_{10}H_{20}O_8$, which was tentatively equated with a hydroxydecanoic acid, and has now been identified as D-3-hydroxydecanoic acid.

Molecular formula of serratamic acid

Earlier experiments favoured a molecular formula $C_{13}H_{25}O_5N$ for serratamic acid but did not entirely exclude the possibility of its being a larger molecule. Subsequently serratamic acid has been converted