Adenosine has been found to form a substrate for regeneration of the essential energy stores of the red cell, even after prolonged incubation when it appears that glucose can no longer be utilized.

6. An attempt has been made to explain some of the biological variations in the red cells of certain mammalian species on a rational basis, and to intimate the importance of metabolism in the storage of red cells and their diseased states.

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The Amino Acid Sequence in Bacitracin A

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Bacitracin A is the major constituent of a family of antibacterial polypeptides known as bacitracin (Craig, Gregory & Barry, 1949; Newton & Abraham, 1950). It has a molecular weight of about 1470 and has been reported to yield the following amino acids on hydrolysis: D-ornithine (Orn) (1), L-lysine (1), L-histidine (1), DL-aspartic acid (2), D-glutamic acid (1), L-cysteine (1), D-phenylalanine (1), L-

leucine (1), L-isoleucine (2) (Craig, Weisiger, Hausmann & Harfenist, 1952; Craig, Hausmann & Weisiger, 1952, 1953; Newton & Abraham, 1953*a,b*). In addition, it yields a significant amount of a substance which runs slightly faster than isoleucine on columns of Dowex 50 (Craig, Hausmann & Weisiger, 1952). This substance has been detected independently by Porath (private communication)

and in this laboratory. Piez (1954) has suggested that it is D-alloisoleucine. If this is so, it appears to be formed in considerably less than one molecular proportion. However, in a publication that became available after the present work had been carried out, Craig, Hausmann & Weisiger (1954) state that they have been forced to the conclusion that bacitracin *A* contains three isoleucine residues instead of two. Suggestions have also been made that bacitracin *A* contains an uncharacterized component which is not an amino acid (Craig *et al.* 1953; Porath, 1953).

An open polypeptide chain composed only of the amino acids known to be present in the molecule would have four free carboxyl groups, three free amino groups, and a fourth basic group due to the glyoxaline ring of histidine. Bacitracin *A* was found to contain only two free carboxyl groups and only three free basic groups, the ϵ -amino group of the lysine residue showing no reaction with 1:2:4-fluorodinitrobenzene (Newton & Abraham, 1953*b*; Craig *et al.* 1953). Newton & Abraham (1953*b*) suggested that one of the masked carboxyl groups might be present as an amide, while the other might be engaged, directly or indirectly, in ring formation with the masked amino group of lysine.

Two of the three basic groups of bacitracin *A* were accounted for by the glyoxaline ring of histidine and the δ -amino group of ornithine. The third basic group titrated in the region expected of the α -amino group of an N-terminal amino acid residue (Newton & Abraham, 1953*b*) and apparently behaved like an amino group in the Van Slyke determination of amino nitrogen (Craig *et al.* 1953). However, Ingram (1953) failed to demonstrate the presence of an N-terminal amino acid in bacitracin *A* by reductive methylation and Porath (1953), using the methods of Sanger & Thompson (1953) and Edman (1950), respectively, obtained neither a dinitrophenyl (DNP) derivative nor a phenylthiohydantoin corresponding to an N-terminal amino acid. Newton & Abraham (1953*a*) and Lockhart, Newton & Abraham (1954) reported that the DNP derivative of bacitracin *A* appeared to give DNP-isoleucine on hydrolysis, but that the yield was much less than that expected from one N-terminal isoleucine residue per mole. The nature of the third basic group in bacitracin *A* therefore remained uncertain.

Bacitracin *A* was also found to contain a latent thiol group. This group, forming part of a cysteine residue, was rapidly unmasked when the antibiotic was treated with N hydrochloric acid at 100°, and its liberation was accompanied by the disappearance of an absorption band at 254 m μ . (Newton & Abraham, 1953*a*; Craig, Weisiger, Hausmann & Harfenist, 1952). Newton & Abraham (1953*b*)

discussed the possibility that the sulphur atom formed part of a thiazoline ring.

These facts suggested that bacitracin *A* was a polypeptide with unusual structural features. Further investigation of its structure appeared to fall into two related parts: the determination of the amino acid sequence in the polypeptide chain, and the determination of the way in which this chain is utilized to form the specific ring systems present in the molecule.

Lockhart *et al.* (1954) reported that the sequence

occurred in bacitracin *A*. The present paper describes further work on the amino acid sequence in bacitracin *A*. Some of the work, however, was carried out with material which contained bacitracin *B* as well as bacitracin *A*. In addition to the amino acids obtained from bacitracin *A*, hydrolysis of bacitracin *B* gives an amino acid which behaves like valine on paper chromatograms (Craig, Weisiger, Hausmann & Harfenist, 1952; Newton & Abraham, 1953*a*). An indication was obtained of the position of the 'valine' residue in the amino acid sequence of bacitracin *B*. The structure of this amino acid is uncertain, since it was isolated by Craig, Weisiger, Hausmann & Harfenist (1952) and reported to be different from valine.

METHODS

The methods used in the present work were similar to those employed by Sanger & Tuppy (1951) and by Sanger & Thompson (1953) for determining amino acid sequences. Peptides formed on partial hydrolysis were separated by ionophoresis in a four-compartment cell (Synge, 1951) and then by chromatography and ionophoresis on paper. The amino acid residues in the peptides were determined by complete hydrolysis and the N-terminal residues by reaction with 1:2:4-fluorodinitrobenzene (FDNB).

Antibacterial activity

Antibacterial activities were expressed in International Units (Humphrey, Lightbown, Mussett & Perry, 1953). The method of assay was similar to that of Brownlee *et al.* (1948). *Corynebacterium xerosis* was used as the test organism (Newton & Abraham, 1953*a*).

Bacitracin used for hydrolysis

Two samples of bacitracin (lot 311 and lot 25) were kindly supplied by the Commercial Solvents Corporation, Terre Haute, Indiana, U.S.A. Lot 311 (45 i.u./mg.) appeared to consist of bacitracins *A* and *B* together with smaller amounts of bacitracins *C*, *D*, *E* and *F* (Newton & Abraham, 1953*a*). Lot 25 (61 i.u./mg.) probably consisted mainly of bacitracin *A*, together with some bacitracin *B*, and possibly *D*. Its ultraviolet absorption spectrum in the region 230–

320 $\mu\mu$. ($\lambda_{\max}$ 254 $\mu\mu$.; $E_{1\%}^{1\text{cm}}$ 25) was similar to that of bacitracin A. Lot 311 was used for preparing bacitracin A. Experiments were also carried out on lot 311 and lot 25 without further purification.

In some cases the latent thiol group in bacitracin was oxidized to $-\text{SO}_3\text{H}$ before hydrolysis. Lot 311 was also inactivated by acid and the product converted into an *S*-methyl derivative, and lot 25 was inactivated by acid and then desulphurized by treatment with Raney nickel. It was intended in this way to obviate difficulties which might otherwise arise from the uncontrolled oxidation of the thiol group during the experiments. It was also thought that changes in the potential cysteine residue might facilitate the identification of some of the peptides formed on hydrolysis, or that they might be accompanied by other changes which would help to throw light on the structure of the molecule.

Bacitracin A. Bacitracin A was prepared by counter-current distribution of lot 311 (6 g.) in a solvent system consisting of isoamyl alcohol-*n*-butanol (4:1, by vol.) and 0.005M potassium phosphate buffer pH 6.8 (Newton & Abraham, 1950). The distribution was carried out in eleven separating funnels (numbered 0-10), using 150 ml. of each phase of the system. The lower (aqueous) phase was mobile. After the 7th fundamental transfer, no new lower layer was added to the first funnel, and the upper layer was discarded, since it contained less than 2% of bacitracin A (Newton & Abraham, 1950). Similar discards were made after the 9th, 11th, 13th, 17th, 24th and 26th transfers to reduce the labour involved in making transfers by hand. On the 11th transfer the first member of the withdrawn series was removed. The distribution was stopped when nineteen withdrawn fractions had been collected and only three funnels of the fundamental series remained.

Photometric ninhydrin estimations of the material in the various fractions and estimations of the distribution of antibacterial activity were made by the methods of Newton & Abraham (1950). The optical densities of the fractions of the withdrawn series (diluted 1:3 with ethanol) were measured at 254 $\mu\mu$. in a Beckman model DU spectrophotometer.

The ratio of antibacterial activity to ninhydrin colour density, or to the optical density at 254 $\mu\mu$., was highest, and nearly constant, for the fractions withdrawn during transfers 23 to 30. The partition coefficient of the material in these fractions, which was estimated from the optical densities at 254 $\mu\mu$., using the formula of Newton & Abraham (1952), was 1.07.

The fractions withdrawn during transfers 23-30 were pooled and concentrated in the manner described by Newton & Abraham (1950). The concentrate (90 ml., pH 4.0) was then freeze-dried, yielding 700 mg. of product with an activity of 72 i.u./mg.

This preparation of bacitracin A showed an absorption maximum at 254 $\mu\mu$. ($E_{1\%}^{1\text{cm}}$ = 21.4). After hydrolysis with 6N-HCl at 105°, a paper chromatogram run with butanol-acetic acid (Woiwod, 1949) showed the typical amino acid pattern of bacitracin A. Only a trace of 'valine' was detected, indicating that very little bacitracin B was present.

***S*-Methyl derivative of acid-inactivated bacitracin.** Bacitracin (500 mg., lot 311) was dissolved in 0.5N-HCl (10 ml.) and the solution heated at 100° for 20 min. The solution was freeze-dried and the residue dissolved in liquid NH_3 (about 30 ml.). Sodium was added in small pieces until the solution developed a permanent blue colour. Methyl iodide (0.1 ml.) was then added and the NH_3 allowed to evaporate. The residue was dissolved in water (25 ml.) and inorganic salts

were removed by ionophoresis in the four-compartment cell. The solution from the specimen compartment (pH 6.5) was freeze-dried, yielding a white powder (125 mg.). After complete hydrolysis of this material with 6N-HCl at 105° for 24 hr., a paper chromatogram run with butanol-acetic acid showed that the spot due to cystine was absent, and a new spot due to *S*-methyl cysteine (R_F = 0.39) had appeared.

Desulphurized acid-inactivated bacitracin. Bacitracin (493 mg., lot 25) was inactivated by acid as in the preceding experiment, and then treated with Raney nickel as described by Newton & Abraham (1953b). The product (400 mg.) was contaminated with inorganic material. It was placed in the specimen compartment of a four-compartment cell (Synge, 1951) and subjected to ionophoresis (the pH being maintained at 6.5) until the current fell to 33 ma at 500 v. The solution was centrifuged to remove a small precipitate of alumina and then freeze-dried. The residue weighed 216 mg. After complete hydrolysis of this material with 6N-HCl at 105° for 24 hr., a paper chromatogram run with butanol-acetic acid (Woiwod, 1949) showed the typical amino acid pattern of bacitracin, except that the spot due to cystine was greatly diminished and a new spot due to alanine had appeared.

Oxidation of the sulphur in bacitracin to $-\text{SO}_3\text{H}$. This was carried out with performic acid, using the method of Sanger (1949).

Partial hydrolysis

Samples of bacitracin were hydrolysed in 11N-HCl in sealed tubes at 37° (Sanger & Tuppy, 1951) for times varying from 1 to 10 days.

The photometric ninhydrin reaction (Moore & Stein, 1948) was used to make an approximate estimate of the average chain length of the resulting peptides. The average chain length was calculated from the amount of colour developed, a correction being made to allow for the fact that one mole of NH_3 was probably liberated at an early stage of the hydrolysis. Bacitracin A behaves as though it contained a labile amide group in addition to eleven or twelve amino acid residues. When preparations of desulphurized, acid-inactivated bacitracin or the *S*-methyl derivative of acid-inactivated bacitracin were used, which no longer contained 'amide' nitrogen, no correction was necessary.

Preliminary experiments showed that the average chain length fell rapidly to 4 in 24 hr. and then slowly decreased to 2.5 in 6 days and 2.0 in 10 days.

Separation of the peptides

Ionophoresis in a four-compartment cell. A preliminary fractionation of the peptides formed on partial hydrolysis of about 100 mg. of material was usually carried out in a four-compartment cell similar to that described by Synge (1951). Strongly basic peptides migrated into the cathode compartment, neutral peptides remained in the specimen compartment, and strongly acidic peptides migrated into the acetic acid compartment.

One-dimensional paper chromatograms. The solutions from the four-compartment cell were evaporated separately to dryness *in vacuo* and each residue dissolved in about 0.1 ml. water. Chromatograms of the resulting solutions were run with butanol-acetic acid (Woiwod, 1949) on separate sheets of Whatman no. 1 paper. Twenty spots of a solution (each 5-6 μl .) were placed on a sheet of paper at 12.5 mm. intervals, and on either side of them two spots were placed 25 mm. further away. After running the

chromatogram, vertical strips which contained the material from the outer spots were cut off each side of the paper. These strips were sprayed with ninhydrin solution and heated at 105° for 10 min. The resulting pattern served as a guide, which enabled the major portion of the chromatogram to be cut into horizontal strips carrying peptides and amino acids which had been separated from each other. These substances were eluted from the strips with water, using an apparatus similar to that of Kemble & Macpherson (1954) except that the trough was of stainless steel. The eluates were allowed to drop into Pyrex tubes (1.0 × 7.5 cm.) and evaporated to dryness *in vacuo*. In some cases, in which the peptides were not sharply resolved, it was found that a clearer separation could be obtained by subjecting the separate eluates to paper chromatography, using butanol-acetic acid.

Ionophoresis on paper. In most cases, the homogeneity of the peptides obtained from paper chromatograms was tested by ionophoresis at pH 7.0 on sheets of Whatman no. 1 paper 36 cm. long, in an apparatus similar to that of Flynn & Mayo (1951). The buffer was prepared by adding 36.3 ml. of commercial collidine (distilled under N_2 ; b.p. 54°/12 mm.) and 185 ml. of *N* acetic acid to 3.86 l. of water (Newton & Abraham, 1954). If a fraction was shown to consist of more than one component by ionophoresis, 10 spots (each 7.2 μ l.) were placed at 12.5 mm. intervals on a sheet of paper together with a further spot at each end to act as a guide. After 500 v had been applied to the ends of the paper for 2.5 hr., the guide strips were developed with ninhydrin and the position of the resulting spots enabled the separate components from the main series of spots to be eluted in the manner used with paper chromatograms.

Other procedures. In a few cases the methods used were different from those described above. They included two-dimensional paper chromatography and ionophoresis on paper at pH 3.

Two-dimensional paper chromatograms were run first with butanol-acetic acid (Woiwod, 1949) and then with a collidine-lutidine mixture (Dent, 1948), using about 1 mg. of peptide (in 6.3 μ l.) on each of four sheets of Whatman no. 3 MM paper. One of the sheets was sprayed with ninhydrin and the pattern obtained was used to estimate the position of the peptides on the untreated sheets. The areas of paper round these positions were cut out and the peptides eluted with water.

Ionophoresis on paper at pH 3 was carried out in 0.05 *N* acetic acid for 5 hr., using a potential gradient of 14 v/cm. This procedure was used with peptide mixtures in which the sulphur had been oxidized to $-SO_3H$, in the hope that highly acidic peptides containing cysteic acid would be readily separated from the remainder (Sanger & Thompson, 1953).

Analysis of peptides

Peptides formed on partial hydrolysis which appeared to have been obtained in a homogeneous form were hydrolysed in 6 *N*-HCl at 110° for 20 hr. The resulting amino acids were determined by chromatography and ionophoresis on paper. Most of the amino acids present in bacitracin could easily be distinguished on chromatograms run with butanol-acetic acid (Woiwod, 1949). Lysine was distinguished from ornithine by ionophoresis on paper for 2.5 hr. in the collidine acetate buffer (pH 7.0) previously described, using a potential gradient of 14 v/cm. on sheets of Whatman no. 1 paper 36 cm. long. Ornithine migrated slightly faster towards the cathode than lysine, and provided that the

amount of each of these amino acids was less than 4 μ g. and the volume of the solution applied to the paper did not exceed 2 μ l., two distinct spots were formed when both were present. Histidine migrated about one-third as far as lysine. Leucine was well separated from isoleucine on paper chromatograms by using the *tert*-amyl alcohol-water system of Work (1949). However, there is no evidence that isoleucine would have been separated from *alloisoleucine*.

In early experiments, *N*-terminal amino acids in the peptides were determined by the method of Sanger & Thompson (1953). Later this method was modified in an attempt to reduce the formation of 2:4-dinitrophenol, by using trimethylamine carbonate instead of trimethylamine. The peptide (50–150 μ g.) was dissolved in 0.1 ml. of a 1.5% (w/v) solution of trimethylamine carbonate (pH 9.3), FDNB (0.01 ml.) in ethanol (0.2 ml.) was added, and the reaction allowed to proceed in the dark for 2.5 hr. The ethanol was removed *in vacuo*, 0.24 ml. of 1% (w/v) trimethylamine carbonate was added and excess FDNB extracted from the resulting solution with ether. The aqueous solution was evaporated to dryness *in vacuo*, the residue dissolved in 0.1 ml. of 6 *N*-HCl and the solution heated in a Pyrex tube under N_2 for 9 hr. at 105°. The hydrolysate was diluted with 2 vol. of water and the resulting solution shaken 3 times with an equal vol. of diethyl ether to extract the ether-soluble DNP-amino acids. Di-DNP-histidine (if present) was extracted with *n*-butanol. The extracted DNP-amino acids were run on paper chromatograms, using the system of Blackburn & Lowther (1951). The amino acids and any water-soluble DNP derivatives were studied on paper chromatograms run with butanol-acetic acid and by ionophoresis on paper at pH 7.0.

RESULTS

The results obtained from the various experiments are shown in Tables 1–4 and Fig. 1. In Fig. 1 the results obtained when mixtures of peptides were

Fig. 1. Resolution of peptides from bacitracin A by chromatography and ionophoresis on paper (see Table 1). The central vertical line indicates the position of substances that have no net charge at pH 7.

run on paper chromatograms, eluted, and then subjected to ionophoresis on paper, have been brought together in a single diagram. The abbreviations used for amino acids are those recommended by Brand & Edsall (1947) and the methods of writing peptide structures are those of Sanger & Tuppy (1951). When leucine has not been distinguished from isoleucine the symbol 'Leu' is used.

Column 2 shows the R_f values on paper chromatograms run with butanol-acetic acid. Column 3 shows the distance which the spots moved during ionophoresis on paper. Unless otherwise stated, this was carried out at pH 7.0 for 2.5 hr. with a potential of 500 v in the manner previously described. Distances from the origin towards the cathode are given a negative sign. Substances with no net charge at pH 7.0 were carried about 1 cm. towards the cathode by endosmosis. Column 4 of the tables shows the amino acids detected on complete hydrolysis and the X's in column 5 indicate their approximate strength. Column 6 shows the relative strength of the amino acids remaining in the aqueous solution after the extraction of the hydrolysed DNP-peptide solution with diethyl ether and, in the case of peptides containing histidine, *n*-butanol. Column 7 shows the DNP-amino acid identified in the extract. In column 9, which contains cross references to apparently identical peptides, the first figure indicates the number of the table in which the peptide occurs, and subsequent figures the spot number.

A number of the peptides formed on partial hydrolysis of bacitracin appeared to yield cysteic acid on complete hydrolysis even though they came from material which had not been oxidized with performic acid. Some oxidation of the —SH group to an —SO₃H group occurred in the four-compartment cell, in which the solutions were stirred by a stream of air. The reason why the oxidation proceeded beyond the —S—S— stage is not clear; this was never observed with the mixture of amino acids formed by complete hydrolysis of bacitracin.

Experiment 1. Peptides from bacitracin A

Bacitracin A was hydrolysed with 11 N-HCl for 6 days at 37°, the HCl was removed *in vacuo*, and the partial hydrolysate was oxidized with performic acid. The product was separated into acidic, neutral, and basic fractions in the four-compartment cell and the peptides in these fractions were further resolved by paper chromatography and subsequent ionophoresis on paper at pH 7.0 (Fig. 1). In other experiments the oxidation with performic acid was omitted, or was carried out before hydrolysis.

The peptides are listed in Table 1. Peptides 1–18, 20–22, and 24 were obtained from bacitracin A oxidized after partial hydrolysis, peptide 25 was obtained from bacitracin A oxidized before hydrolysis, and peptides 19 and 23 were obtained from unoxidized material. Peptides 1–5, 8–11 and 18–19 were obtained from the cathode compartment of the

four-compartment cell, peptides 6, 12 and 23 from the specimen compartment, and peptides 7, 13–17, 20–22, and 24 and 25 from the acetic acid compartment.

Experiment 2. Peptides from commercial bacitracin

Peptides obtained from a sample of commercial bacitracin (lot 311, 45 i.u./mg.) and from the same material oxidized with performic acid are listed in Table 2. Partial hydrolysis was effected in both cases with 11 N-HCl for 6 days at 37°.

Peptides 1–13 and 18 were obtained from the unoxidized sample of bacitracin. Peptides 7, 8, 9, 11 and 13 were obtained from paper chromatograms of the acidic, neutral, and basic fractions from the four-compartment cell. Peptides 1–6 were obtained by the same procedure followed by ionophoresis on paper at pH 7.0. This readily separated 1 from 2. Peptides 10 and 12 were obtained by oxidizing the neutral fraction from the four-compartment cell with performic acid and subjecting the product to further ionophoresis in the four-compartment cell. Peptide 18 was obtained by subjecting a sample of the hydrolysate to ionophoresis on paper at pH 7.0.

Peptides 14–17 and 19–21 were obtained from the oxidized sample of bacitracin. Part of the hydrolysate was subjected to ionophoresis in the four-compartment cell and the resulting acidic fraction yielded peptides 14–17 on a paper chromatogram. The remainder of the hydrolysate was subjected to ionophoresis on paper in 0.05 N acetic acid, when all the peptides migrated towards the cathode except some containing cysteic acid. Spots which behaved as neutral or acidic were eluted and yielded peptides 19–21 on a paper chromatogram.

Experiment 3. Peptides from an S-methyl derivative of acid-inactivated bacitracin

Peptides obtained from the *S*-methyl derivative of acid-inactivated bacitracin (lot 311, 45 i.u./mg.) after hydrolysis with 11 N-HCl at 37° for 10 days are listed in Table 3. The cysteine residue in bacitracin had been converted into *S*-methyl cysteine, which was easily identified on a paper chromatogram run with butanol-acetic acid.

The hydrolysate was separated into neutral, basic, and acidic fractions in the four-compartment cell and the peptides in these fractions were further resolved by paper chromatography and by ionophoresis on paper at pH 7.0. Peptides 1–10 came from the neutral fraction, 11 from the basic fraction, and 12 from the acidic fraction.

Experiment 4. Peptides from desulphurized acid-inactivated bacitracin

Peptides obtained from desulphurized acid-inactivated bacitracin (lot 25; 61 i.u./mg.), after hydrolysis with 11 N-HCl for 8.5 days, are listed in Table 4. The hydrolysate was separated into acidic, neutral, and basic fractions in the four-compartment cell and the peptides in these fractions were resolved by paper chromatography and subsequent ionophoresis on paper at pH 7.0. Peptides 1–7 were obtained from the specimen compartment of the four-compartment cell, 8 from the acetic acid compartment and 9–14 from the cathode compartment.

Table 1. *Peptides from bacitracin A*

Spot no. (Fig. 1)	R_f value (butanol-acetic acid)	Migration (pH 7.0) in cm.	Amino acids present	Strength of amino acid after		DNP-amino acid identified	Structure*	Identical with†
				Hydrolysis	DNP treatment			
1	0.055	-2.2	{ His Asp Lys Orn 'Leu'	x x x x x x x x x ?	- x x x x - -	His	His.[Asp, Asp, Lys, Orn]	.
2	0.11	-12.0	Orn	x x x	.	.	Ornithine	2.2 & 3.11
3	0.095	-11.0	{ Lys Orn 'Leu'	x x ?	. . .	.	[Lys, Orn]	.
4	0.10	-5.3	{ Asp Lys Orn 'Leu'	x x x x x x x	- x x x † -	.	Asp.[Lys, Orn, 'Leu']	2.1 & 3.1
5	0.13	-4.5	His	x x x	.	.	Histidine	2.8
6	0.14	5.0	{ His Asp	x x x x x	- x x	.	His. Asp, or His. Asp, Asp	3.3
7	0.07	13.5	CySO ₂ H	x x x	.	.	Cysteic acid	2.21
8	0.21	-1.0	{ Asp Lys Orn 'Leu'	x x x x x x x x		.	[Asp, Asp, Lys, Orn, 'Leu']	.
9	0.26	-7.0	{ Lys Orn 'Leu'	x x x x x x	- x † x	.	Lys.[Orn, 'Leu']	.
10	0.27	-7.0	{ Orn 'Leu' His Phe	x x x x x x ? .	x † x x - -	Orn	Orn, 'Leu'	.
11	0.28	-5.0	{ Phe His Lys Orn 'Leu'	x x x x x x x .		.	[Phe, His]	3.4 & 4.4
12	0.26	3.0	{ Asp Glu Lys Orn 'Leu'	x x x x x x	x x x x x † x	Asp &/or Glu §	[Asp, Asp, Glu, Lys, Orn, 'Leu']	.

13	0.29	3.0	{ Asp Glu Lys Ileu	{ x x x x x x x x x	{	[Asp, Glu, Lys, Ileu]	.
14	0.29	6.5	{ CySO ₃ H 'Leu' Glu Lys Asp	{ x x x x x x x x x x	{	[CySO ₃ H, 'Leu', Glu, Lys, Asp]	.
15	0.21	11.0	Asp	x x x x	.	Aspartic acid	2.11
16	0.28	10.0	Glu	x x x	.	Glutamic acid	2.14
17	0.41	13.0	{ CySO ₃ H Leu Glu	{ x x x x x x x x x	{ x x x x x x	CySO ₃ H.[Leu, Glu]	2.19
18	0.48	-5.5	{ Orn Ileu Unknown (P)	{ x x x x x x x x x x x	{ x † x x x x x	Orn.[Ileu, P]	.
19	0.48	-5.5	{ Orn Ileu Phe	{ x x x x x x x x x x	{ - x x x x	Orn.[Ileu, Phe]	2.6, 3.7 & 4.14
20	0.55	6.0	{ Leu Glu	{ x x x x x x x x	{ x x x x	Leu, Glu	2.13, 3.12 & 4.8
21	0.50	9.0	{ CySO ₃ H Leu	{ x x x x x x x x	{ x x x x x	CySO ₃ H, Leu	2.16 & 2.20
22	0.63	-1.0	Ileu	x x x x	.	Isoleucine¶	3.8
23**	0.68	-1.0	{ Phe 'Leu'	{ x x x x x x x x	{ x x x x x x x	Phe, 'Leu'	.
24	0.75	3.3	{ Asp Glu Lys 'Leu'	{ x x x x x x x x	{ x - x x x	Glu.[Asp, Lys, 'Leu']††	.
25	0.58	.	{ 'Leu' CySO ₃ H Glu	{ x x x x x x x x x x	{ x x x x x x x x x	'Leu', [CySO ₃ H, 'Leu', Glu]	2.17

* Square brackets indicate that the sequence of amino acids inside the brackets has not been determined.

† The first figure indicates the number of the table in which the peptide occurs and the subsequent figures the spot no.

‡ Identified as δ -DNP-ornithine.

§ The DNP-amino acid was accompanied by material which remained at the origin on paper chromatograms (Blackburn & Lowther, 1951) and showed a pale green fluorescence in ultraviolet light (365 m μ).

|| On paper chromatograms run with butanol-acetic acid and developed with ninhydrin this substance appeared as a grey-blue spot (R_f 0.69) below leucine. The colour was similar to that given by phenylalanine.

¶ This isoleucine came from the acidic fraction from the four-compartment cell. It may have been formed by subsequent hydrolysis of an acidic peptide.

** This peptide was resolved on a two-dimensional chromatogram using butanol-acetic acid and collidine-lutidine (Dent, 1948). R_f 0.33 in collidine-lutidine.

†† The relationship of this peptide to that forming spot 13 and the reason for its high R_f value are uncertain.

Table 2. *Peptides from commercial bacitracin*

Spot no.	R_f value (butanol-acetic acid)	Migration (pH 7.0) in cm.	Amino acids present	Strength of amino acid after		DNP-amino acid identified	Structure	Identical with
				Hydrolysis	DNP treatment			
1	0.10	-5.3	{ Asp Lys Orn 'Leu'	x x x x x x x x	- x x x* x	Asp	Asp. [Lys, Orn, 'Leu']	1.4 & 3.1
2	0.11	-12.0	Orn	x x x x	.	.	Ornithine	1.2 & 3.11
3	0.36	-7.0	{ Lys Orn 'Leu'	x x x x x x	.	.	[Lys, Orn, 'Leu']	.
4	0.40	-7.0	{ Orn 'Leu' Phe His	x x x x x x x x	.	.	[Orn, 'Leu', Phe, His]	.
5	0.42	-5.5	{ Orn Val Phe	x x x x x x x x x	.	.	[Orn, Val, Phe]	.
6	0.48	-5.5	{ Orn 'Leu' Phe	x x x x x x x x x x x x	- x x x x	Orn	Orn. ['Leu', Phe]	1.19, 3.7 & 4.14
7	0.64	.	None†	.	.	.	.	.
8	0.13	-4.5	His	x x x x	.	.	Histidine	1.5
9	0.63	.	{ Phe 'Leu' CySO ₃ H	x x x x x x x x x x	.	.	[Phe, 'Leu', CySO ₃ H]‡	.
10	0.65	-1.0	{ Phe 'Leu'	x x x x x x	.	.	[Phe, 'Leu']	.
11	0.21	11.0	Asp	x x x x	x	Asp	Aspartic acid	1.15
12	0.34	.	{ CySO ₃ H 'Leu'	x x x x x x x	.	.	[CySO ₃ H, 'Leu']	.

13	0.55	6.0	{ Leu Glu	{ x x x x x x	{ x x x x x x	Leu	Leu, Glu	1.20, 3.12 & 4.8
14	0.28	10.0	Glu	x x x	.	.	Glutamic acid	1.16
15	0.36	.	{ 'Leu' CySO ₃ H	{ x x x x x x	{ x x x x x x	{ 'Leu' CySO ₃ H	{ 'Leu', CySO ₃ H	.
16	0.50	9.0	{ CySO ₃ H 'Leu'	{ x x x x x x	{ x x x x x x	{ CySO ₃ H	CySO ₃ H, 'Leu'	1.21 & 2.20
17	0.58	.	{ CySO ₃ H Glu 'Leu'	{ x x x x x x x x x	{ x x x x x x x x x	{ 'Leu'	{ 'Leu', [CySO ₃ H, 'Leu', Gln]	1.25
18	0.28	-3.6	{ His Asp Lys Orn 'Leu'	{ x x x x x x x x x x	{	.	[His, Asp, Lys, Orn, 'Leu']	.
19§	0.41		{ CySO ₃ H 'Leu' Glu	{ x x x x x x x x x	{ - x x x x	{ CySO ₃ H	CySO ₃ H, [Leu, Glu]	1.17
20	0.50		{ CySO ₃ H Leu	{ x x x x x x	{ - x x x x	{ CySO ₃ H	CySO ₃ H, Leu	1.21 & 2.16
21	0.07		CySO ₃ H	x x	.	.	Cysteic acid	1.7

* Identified as δ-DNP-ornithine.
† This spot gave no colour with ninhydrin but showed a pale blue fluorescence in ultraviolet light (365 mμ.). It yielded no amino acids on hydrolysis with 6N-HCl at 110°.
‡ This peptide appeared to yield cysteic acid on hydrolysis, although it came from the specimen compartment of the four-compartment cell. Oxidation of the sulphur may have occurred in subsequent manipulations.
§ This spot gave initially with ninhydrin a yellow colour, which later changed to purple.
|| These peptides were subjected to ionophoresis at pH 3 on Whatman no. 3 MM paper (36 cm. long) at 500 v for 5 hr. 19 and 20 migrated 1 cm. towards the cathode and 21 migrated 11.5 cm. towards the anode.

Table 3. *Peptides from an S-methyl derivative of acid-inactivated bacitracin*

Spot no.	R_f value (butanol-acetic acid)	Migration (pH 7.0) in cm.	Amino acids present	Strength of amino acid after		DNP-amino acid identified	Structure	Identical with
				Hydrolysis	DNP treatment			
1	0.10	-5.3	{ Asp Lys Orn Ileu }	{ x x x x x x x }	{ x x x x x* x }	Asp	Asp. [Lys, Orn, Ileu]	1.4 & 2.1
2	0.09	-1.0	{ Asp Glu Lys Orn Ileu }	{ x x x x x x x x x x }	{ x - x x x* x }	Asp & Glu	Asp. (Glu). [Lys, Orn, Ileu.]	4.3
3	0.14	5.0	{ His Asp }	{ x x x x }	{ - x x }	His	His. Asp, or His. Asp. Asp	1.6
4	0.28	-5.0	{ Phe His }	{ x x x x }	{ . .	.	[Phe, His]	1.11 & 4.4
5	0.37	-7.5	{ Lys Orn Ileu }	{ x x x x x x }	{ - - x x }	.	[Lys, Orn, Ileu]	.
6†	0.38	-2.0	{ Asp Lys Ileu }	{ x x x x x x }	{ - x x - }	Asp‡	Asp. (Ileu). Lys	.
7	0.48	-5.5	{ Orn Ileu Phe }	{ x x x x x x }	{ ?* x x x x }	Orn	Orn. [Ileu, Phe]	1.19, 2.6 & 4.14
8	0.63	-1.0	Ileu	x x x	.	.	Isoleucine	1.22
9	0.72	-1.0	{ Ileu Phe CysCH ₃ }	{ x x x x x x x x x }	{ x x x x x x }	Ileu	Ileu. [Phe, Ileu, CysCH ₃]	.
10	0.79	-1.0	{ Ileu CysCH ₃ Leu }	{ x x x x x x x x x }	{ x x x x x x }	Ileu	Ileu. [CysCH ₃ , Leu]	.
11	0.11	-12.0	Orn	x x x	.	.	Ornithine	1.2 & 2.2
12	0.55	6.0	{ Leu Glu }	{ x x x x x x }	{ x x x x }	Leu	Leu. Glu	1.20, 2.13 & 4.8

* Identified as δ -DNP-ornithine.

† This spot gave a yellow-brown colour with ninhydrin.

‡ The DNP-aspartic acid was accompanied by material which remained at the origin on a paper chromatogram (Blackburn & Lowther, 1951) and showed a bright green fluorescence in ultraviolet light (365 m μ). A trace of material which behaved like DNP-isoleucine was also present.

DISCUSSION

In so far as their composition could be ascertained by the use of paper chromatography, almost all the peptides obtained from bacitracin *A* and from the two commercial samples of bacitracin were consistent with the same amino acid sequence, even though the commercial samples contained some bacitracin *B* and other bacitracins. The different bacitracins show strong similarities in the amino acid patterns given by their hydrolysates on paper chromatograms (Newton & Abraham, 1953*a*) and it may be that they also resemble each other in the order of amino acids in the peptide chains.

Certain peptides were frequently encountered in relatively large amounts in different experiments, particularly, leucylglutamic acid, β -sulphoalanyl-leucylglutamic acid, β -sulphoalanylleucine and ornithyl[isoleucylphenylalanine]. Other peptides, especially those containing histidine and aspartic acid, or having glutamic acid as an N-terminal residue, were relatively rare. Free histidine and free aspartic acid appeared early during the hydrolysis. These facts indicated that there were preferential points of cleavage in the molecule.

Since bacitracin *A* contains only one leucine residue, the peptides Leu.Glu (Table 1, spot 20) and $\text{CySO}_3\text{H}.\text{Leu}$ (spot 21) indicate that peptide 17 is $\text{CySO}_3\text{H}.\text{Leu}.\text{Glu}$. Peptide 25 should therefore be Ileu.CySO₃H.Leu.Glu. The peptides Orn.-'Leu' (spot 10) and Orn.[Ileu, Phe] (spot 19) indicate that there is a sequence: Orn.Ileu.Phe. The peptides [Lys, Orn] (spot 3), Lys.[Orn, 'Leu'] (spot 9), Asp.[Lys, Orn, 'Leu'] (spot 4) and [Asp, Asp, Lys, Orn, 'Leu'] (spot 8) allow the latter sequence to be extended to Asp.Asp.Lys.Orn.-Ileu.Phe. The peptides His.Asp (spot 6) and His.[Asp, Asp, Lys, Orn] (spot 1) indicate that the histidine residue is linked to an aspartic acid residue. Hence, these peptides are consistent with the following sequences in bacitracin *A*:

- (1) Ileu.CyS.Leu.Glu
- (2) His.Asp.Asp.Lys.Orn.Ileu.Phe

In the latter sequence it remains uncertain whether one of the aspartic acid residues forms a branch from the main chain.

Many of the lower peptides obtained from partial hydrolysates of the samples of commercial bacitracin (Tables 2 to 4) would fit into these sequences. The peptide [His, Asp, Lys, Orn, 'Leu'] (Table 2, spot 18) suggests that one of the aspartic acid residues may form a branch in the chain. This will be indicated by the symbol (Asp).

The peptides [Asp, Glu, Lys, Ileu] (Table 1, spot 13) from bacitracin *A* and Asp.(Ileu).Lys (Table 3, spot 6) from commercial bacitracin indi-

cated that the two sequences were linked together through an isoleucine residue in the following manner in which $\rightarrow$ denotes a C—N bond:

The peptides [Asp, Asp, Glu, Lys, Orn, 'Leu'] (Table 1, spot 12), [CySO₃H, 'Leu', Glu, Lys, Asp] (Table 1, spot 14) and Glu.[Asp, Lys, 'Leu'] (Table 1, spot 24) from bacitracin *A*, and the peptides Asp.(Glu).[Lys, Orn, Ileu] (Table 3, spot 2) and Asp.('Leu').[Asp, Lys, Orn], Asp.('Leu').[Lys, Orn] and [Asp, Glu, Lys, Orn, 'Leu'] (Table 4, spots 1 to 3) from commercial bacitracin were consistent with such a linkage. This arrangement involves the assumption that there are three isoleucine residues, or potential isoleucine residues, in the molecule. Hence, two sets of peptides could be formed which contained a lysine, ornithine and one isoleucine residue.

There were indications of some peculiarity in the Asp.(Ileu).Lys fragment. After treatment with FDNB and hydrolysis, the peptides [Asp, Asp, Glu, Lys, Orn, 'Leu'] (Table 1, spot 12), Asp.(Ileu).Lys (Table 3, spot 6) and Asp.('Leu').-[Lys, Orn, 'Leu'] (Table 4, spot 9) yielded ether-soluble material which showed a green fluorescent spot at the origin on paper chromatograms when viewed under ultraviolet light (365 m μ .) and which did not correspond to the DNP derivative of a known amino acid. A similar fluorescent spot was obtained on chromatographing the ether-soluble material from a hydrolysate of DNP-bacitracin *A*. Although free glutamic acid, free aspartic acid, and free ornithine were found to be present in partial hydrolysates, free lysine was never observed, and none of the peptides which were allowed to react with FDNB yielded a mono-DNP-lysine on subsequent hydrolysis. These results showed that one or both of the amino groups of lysine were involved in particularly stable linkages.

The peptides [Phe, His] (Table 1, spot 11) from bacitracin *A* and Phe.His (Table 3, spot 4; Table 4, spot 4) and [Orn, 'Leu', Phe, His] (Table 2, spot 4) from commercial bacitracin indicated that histidine and phenylalanine residue should be linked together, in the sequence shown above, to form a ring. The resulting structure would then be identical with that put forward by Craig *et al.* (1954), but different from that suggested by Porath (1953).

One peptide appeared to be Phe.'Leu' (Table 1, spot 23). Others behaved as though they were [Phe, 'Leu', CySO₃H] (Table 2, spot 9), Ileu.[Phe, Ileu, CySCH₃] (Table 3, spot 9) and Ileu.[Phe, Ileu, Ala] (Table 4, spot 7), although the possibility that they were mixtures of dipeptides cannot be

Table 4. *Peptides from desulphurized acid-inactivated bacitracin*

Spot no.	R_f value (butanol-acetic acid)	Migration (pH 7.0) in cm.	Amino acids present	Strength of amino acid after		DNP-amino acid identified	Structure	Identical with
				Hydrolysis	DNP treatment			
1	0.05	-1.0	$\left\{ \begin{array}{l} \text{Asp} \\ \text{Lys} \\ \text{Orn} \\ \text{'Leu'} \end{array} \right\}$	$\begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \times \\ \times \end{array}$	$\left\{ \begin{array}{c} \times \times \\ \times \times \\ \times * \\ ? \end{array} \right\}$	Asp & 'Leu'	Asp. ('Leu'). [Asp, Lys, Orn]	.
2	0.05	-5.2	$\left\{ \begin{array}{l} \text{Asp} \\ \text{Lys} \\ \text{Orn} \\ \text{'Leu'} \end{array} \right\}$	$\begin{array}{c} \times \\ \times \times \\ \times \times \\ \times \end{array}$	$\left\{ \begin{array}{c} ? \\ \times \times \\ \times * \\ ? \end{array} \right\}$	Asp & 'Leu'	Asp. ('Leu'). [Lys, Orn]	.
3	0.09	-1.0	$\left\{ \begin{array}{l} \text{Asp} \\ \text{Glu} \\ \text{Lys} \\ \text{Orn} \\ \text{'Leu'} \end{array} \right\}$	$\begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \times \\ \times \times \times \\ \times \times \end{array}$	$\left\{ \begin{array}{c} . \\ . \\ . \\ . \\ . \end{array} \right\}$	.	[Asp, Glu, Lys, Orn, 'Leu']	3.2
4	0.28	-5.0	$\left\{ \begin{array}{l} \text{Phe} \\ \text{His} \end{array} \right\}$	$\begin{array}{c} \times \times \\ \times \times \end{array}$	$\left\{ \begin{array}{c} - \\ \times \dagger \end{array} \right\}$	Phe	Phe. His	1.11 & 3.4
5	0.30	-4.0	$\left\{ \begin{array}{l} \text{Lys \&/or Orn} \\ \text{'Leu'} \\ \text{Phe} \\ \text{His} \\ \text{Asp} \end{array} \right\}$	$\begin{array}{c} \times \times \\ \times \times \\ \times \times \\ \times \times \\ \times \times \end{array}$	$\left\{ \begin{array}{c} . \\ . \\ . \\ . \\ . \end{array} \right\}$	.	[Lys &/or Orn], 'Leu', Phe, His, Asp]	.
6	0.33	-7.0	$\left\{ \begin{array}{l} \text{Orn} \\ \text{Lys} \\ \text{'Leu'} \end{array} \right\}$	$\begin{array}{c} \times \times \\ \times \times \\ \times \times \end{array}$	$\left\{ \begin{array}{c} . \\ . \\ . \end{array} \right\}$	.	[Orn, Lys, 'Leu']	.
7	0.71	-1.0	$\left\{ \begin{array}{l} \text{Ileu} \\ \text{Phe} \\ \text{Ala} \\ \text{Val} \end{array} \right\}$	$\begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \\ \times \end{array}$	$\left\{ \begin{array}{c} \times \times \\ \times \times \\ \times \\ ? \end{array} \right\}$	Ileu	Ileu. [Phe, Ileu, Ala]	.

8	0.55	6.0	{ Leu Glu	{ x x x x x x x x x	{ x x x x	{ Leu	Leu, Glu	1.20, 2.13 & 3.12
9	0.16	-6.0	{ Asp Lys Orn 'Leu'	{ x x x x x x x x	{ x x x x x*	{ Asp† & 'Leu'	Asp.('Leu'), [Lys, Orn, 'Leu',]	.
10	0.40	-1.0	{ Asp Lys Orn Ileu Phe	{ x x x x x x x x x x x	{	{ .	[Asp, Asp, Lys, Orn, Ileu, Phe]	.
11	0.29	-7.5	{ Lys Orn Val	{ x x x	{ . . .	{ .	[Lys, Orn, Val]	.
12	0.36	-7.5	{ Lys Orn 'Leu'	{ x x x x x x x x x	{ - x x* x x	{ Lys?	Lys, [Orn, 'Leu']	.
13	0.36	-4.3	{ Asp Lys Orn 'Leu' Phe	{ x x x x x x x x x x	{ ? x x x* x x x x	{ .	Asp. [Lys, Orn, 'Leu', Phe]	.
14	0.48	-5.5	{ Orn 'Leu' Phe Val	{ x x x x x x x x x x x	{ x* x x x x ?	{ Orn	Orn, ['Leu', Phe]	1.19, 2.6 & 3.7
15	0.13	-2.3	Unknown§	x x x	.	.	.	.

* Identified as δ-DNP-ornithine.
† Identified as gly-DNP-histidine.
‡ A trace of DNP-aspartic acid was accompanied by material which remained at the origin on a paper chromatogram (Blackburn & Lowther, 1951) and which showed a green fluorescence in ultraviolet light (365 mμ.).
§ This substance gave a positive Pauly reaction and a grey colour (which developed slowly) with ninhydrin. It migrated only half as far as histidine when subjected to ionophoresis on paper at pH 7. It appeared to be unchanged by treatment with 6N-HCl at 110°.

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Biochemical Studies of Toxic Agents

7. THE METABOLISM OF NAPHTHALENE IN ANIMALS OF DIFFERENT SPECIES

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Although from time to time various metabolites have been isolated from the urines of animals dosed with naphthalene, no systematic study of the influence of species on the metabolism of this hydrocarbon has hitherto been made. In the present work the metabolism of naphthalene in rabbits, rats, guinea pigs and mice has been studied. Paper chromatography has been used to examine the urines of the dosed animals and a number of metabolites have also been isolated. The metabolism of the hydrocarbon in guinea pigs and mice had not previously been investigated. Our findings show that although the optical activity of the metabolite 1:2-dihydronaphthalene-1:2-diol is influenced by species, most of the metabolic reactions which naphthalene has been shown to undergo occur in all four of the animal species studied.

CHROMATOGRAPHIC STUDIES

Reference compounds

1- and 2-Naphthol. These compounds were AnalaR reagents obtained from British Drug Houses Ltd., and each was recrystallized once from water before use.

1:2-Dihydroxynaphthalene. Finely powdered 1:2-naphthoquinone (1.0 g.) was stirred into water (15 ml.) and the suspension was warmed on a water bath with constant stirring. The mixture was then cooled to room temperature, centrifuged and the supernatant was discarded. The moist naphthoquinone was added in small amounts to 15 ml. of an aqueous solution of sodium dithionite (10%, w/v) and, when the additions had been completed, the mixture was left in the refrigerator overnight. The tan-coloured precipitate which formed was removed by filtration and dried *in vacuo*. The product was recrystallized twice from light petroleum (b.p. 60–80°) and yielded 0.341 g. of colourless needles, m.p. 104° (m.p.'s reported in this paper are uncorrected).

Fieser & Fieser (1939) have reported that 1:2-dihydroxynaphthalene melts at 104°. (Found: C, 74.8; H, 5.1. Calc. for $C_{10}H_8O_2$: C, 75.0; H, 5.0%.)

1:4-Dihydroxynaphthalene. This was prepared from 1:4-naphthoquinone by the procedure just described. The compound melted at 177°. The m.p. given by Heilbron & Bunbury (1953) is 176°. (Found: C, 74.5; H, 4.8. Calc. for $C_{10}H_8O_2$: C, 75.0; H, 5.0%.)

1:2-Dihydronaphthalene-1:2-diol. Biosynthetic methods were used to obtain ($\pm$)-trans-1:2-dihydronaphthalene-1:2-diol. Naphthalene was administered to male hooded rats and 1:2-dihydronaphthalene-1:2-diol was isolated from the urine by the method described by Young (1947). The compound isolated from the urine of rats of the strain used in the present work showed no measurable optical rotation and it melted at 100°. (Found: C, 74.1; H, 6.1. Calc. for $C_{10}H_{10}O_2$: C, 74.0; H, 6.2%.) The diacetate of the compound showed no optical rotation and melted at 74°. (Found: C, 68.7; H, 5.5. Calc. for $C_{14}H_{14}O_4$: C, 68.3; H, 5.7%.)

1-Naphthyl glucosiduronic acid. The method of Berenbom & Young (1951) was used to isolate this compound from the urine of rabbits dosed with 1-naphthol. The compound so obtained melted at 199°, $[\alpha]_D^{20} - 85^\circ$ in ethanol (c, 1). The triacetyl methyl ester was prepared, m.p. 160°, $[\alpha]_D^{20} - 79^\circ$ in $CHCl_3$ (c, 1). (Found: C, 59.5; H, 5.7; CH_3CO —, 27.7. Calc. for $C_{23}H_{24}O_{10}$: C, 60.0; H, 5.2; CH_3CO —, 28.0%.)

2-Naphthyl glucosiduronic acid. This was isolated in a similar way after rabbits were dosed with 2-naphthol. The compound melted at 153–154°, $[\alpha]_D^{20} - 96^\circ$ in ethanol (c, 1). The triacetyl methylester had m.p. 127–128° and $[\alpha]_D^{20} - 35^\circ$ in $CHCl_3$ (c, 1). (Found: C, 60.4; H, 5.3; CH_3CO —, 27.6. Calc. for $C_{23}H_{24}O_{10}$: C, 60.0; H, 5.2; CH_3CO —, 28.0%.)

Potassium 1-naphthyl sulphate. The method of Feigenbaum & Neuberg (1941) was used in order to synthesize this compound from 1-naphthol. The compound was converted into the *p*-toluidine salt by the procedure described by Barton & Young (1943). Treatment of the *p*-toluidine salt with KOH regenerated the pure potassium salt. (Found: total sulphate S, 12.4. Calc. for $C_{10}H_7O_4SK$, 12.2%.)