A New Method for Degradation of *n*-Carboxylic Acids

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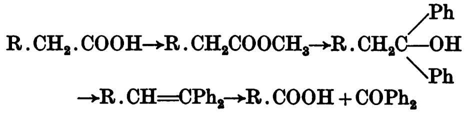
In the course of studies on the mechanism of the biosynthesis of fatty acids, carried out with the aid of isotopically labelled precursors, it became desirable to degrade various long and short chain acids in order to determine the position of the labels in the carbon chains. The methods described in the literature were not considered satisfactory for the scale of work envisaged, although the Barbier-Wieland method (Lane & Wallis, 1941) has been used successfully by Stadtman & Barker (1949) to degrade the hexanoic acid synthesized by *Clostridium kluyveri*. The conversion of the silver salt of a fatty acid under the action of bromine into an alkyl bromide with loss of carbon dioxide (Hunsdiecker & Hunsdiecker, 1942; Oldham, 1950) has also been considered in an alternative scheme. The alkyl bromide might be further degraded to a lower carboxylic acid as shown in Fig. 1.

BARBIER-WIELAND METHOD



$$R.CH_2.COOH \rightarrow R.CH_2COOCH_3 \rightarrow R.CH_2C(OH)Ph_2$$
$$\rightarrow R.CH{=}CPh_2 \rightarrow R.COOH + COPh_2$$

SILVER SALT METHOD

$$R.CH_2CH_2COOH \rightarrow R.CH_2CH_2COOAg \rightarrow R.CH_2CH_2Br(+CO_2)$$
$$\rightarrow R.CH{=}CH_2 \rightarrow R.COOH(+CO_2)$$

Fig. 1. The Barbier-Wieland and silver salt methods of fatty acid degradation.

However, a more than century-old observation was brought to our notice by Dr J. W. Cornforth, which suggested a simpler method of degradation involving simultaneous removal of two carbon atoms as acetic acid from the carboxyl end of the fatty acid. Varrentrap (1840) showed that fusion of oleic acid with solid potassium hydroxide led to the formation of palmitic acid in very good yield, the other main product being acetic acid. Apparently, under the influence of molten potassium hydroxide, the double bond migrated from the 9:10 to the 2:3 ($\alpha\beta$) position before fission of the molecule took place. This observation was confirmed by Edmed (1898), and a few years later a similar reaction was shown to occur when octadec-2-enoic acid was fused with solid potassium hydroxide (Ponzio, 1905). Similarly, decanoic acid has been prepared by the fusion of dodec-2-enoic acid with potassium hydroxide (Kao & Ma, 1931). We have found also that under similar conditions hex-2-enoic acid gave butyric and acetic acid in 80–90 % yield. The method of fatty acid degradation to be described is based on this type of reaction. A preliminary account of some of this work has already been published (Hunter & Popják, 1951).

METHODS AND DISCUSSION

The method of degradation developed involves the following steps: bromination of the carboxylic acid chloride (I), conversion of the brominated product to an ester (II) by a method similar to that used by Blaise & Picard (1912), and dehydrobromination of the ester. The resulting unsaturated ester (III) is then saponified and the free acid fused with potassium hydroxide at 300–340° to yield acetic acid and another saturated carboxylic acid (IV) two carbon atoms shorter than the original acid. These steps may be represented by

$$R.CH_2.CH_2COOH \rightarrow \underset{\text{(I)}}{R.CH_2CH_2COCl} \rightarrow R.CH_2CHBrCOCl$$
$$\rightarrow \underset{\text{(II)}}{R.CH_2CHBr.COOR'} \rightarrow \underset{\text{(III)}}{R.CH{=}CH.COOR'}$$
$$\rightarrow R.CH{=}CH.COOH \rightarrow \underset{\text{(IV)}}{R.COOH} + CH_3COOH.$$

Model experiments were carried out on hexanoic acid and extended to other normal straight-chain acids. It was hoped that it might be possible to effect dehydrobromination and fission in one stage by fusion of α-bromohexanoic acid with solid potassium hydroxide. However, the process gave rise to pentanoic (valeric) acid and to only small quantities of butyric and acetic acids. Attempted direct dehydrobromination of the free α-bromo-acid with collidine or quinoline likewise failed to produce the desired result; only the formation of the corresponding lactide ester was observed, which might have been formed by intermolecular elimination of hydrogen bromide. The dehydrobromination of ethyl α-bromohexanoate had, however, been carried out successfully with quinoline (Fichter & Pfister, 1904) or with diethylaniline (Blaise & Lüttringer, 1905), and attention was therefore turned towards the esters. It was found that methyl hex-2-enoate, prepared from an authentic sample of hex-2-enoic acid, could be degraded directly to butyric and acetic acids by fusion with potassium hydroxide in almost as high a yield as the free acid. It was surprising to find, however, that, in contrast with ethyl α-bromohexanoate, the methyl ester could not be dehydrobrominated under any of the variety of conditions tried, only varying amounts of the lactide ester of α-hydroxyhexanoic acid being obtained. This difference in reactivity of the methyl and ethyl esters suggested a trial of other more complex esters in order to improve the yield from the degradative process.

It seemed likely that a major side reaction causing a reduced yield during dehydrobrominations might occur as a result of nucleophilic attack of freshly liberated bromide ions upon the α-carbon atom of the alcohol participating in the ester grouping. The isolation of some lactide ester in the course of attempted dehydrobromination of the methyl ester of α-bromohexanoic acid provided some evidence for this hypothesis. Accordingly, it became desirable to synthesize esters which would hinder the approach of bromide ions towards the ester grouping. Pursuing these ideas, it was found that the dehydrobromination of *n*-propyl α-bromohexanoate gave a considerably better yield of the ester of the ethenoid acid than the same reaction on the ethyl ester. However, further lengthening of the carbon chain had little more effect, the dehydrobromination of *n*-octyl α-bromohexanoate proceeding in only slightly better yield.

Table 1. *Yields of esters of α-bromohexanoic acid and of hexenoic acid obtained with straight-chain alcohols*

Ester	Yield of α-bromohexanoate (%)	Yield of hexenoate from hexanoic acid (%)
Methyl	68	0
Ethyl	76	11
n-Propyl	81	19
n-Octyl	67	23

Next, the effect on the reactions of the substitution of methyl groups in the α-position of the alcohol was investigated. *iso*Propyl α-bromohexanoate again proved superior to its ethyl analogue, but further substitution was disappointing. The *tert.*-butyl ester of α-bromohexanoic acid formed in only moderate yield from *tert.*-butanol and the dehydrobromination of the product also proceeded unsatisfactorily, presumably due to the elimination of *iso*butene from the ester at the elevated temperature (approx. 180°) necessary for the reaction.

If the yield-reducing process in the dehydrobromination reaction was in fact brought about by the attack of bromide ions, there was a formal analogy here to the extensively studied interaction of nucleophilic reagents with alkyl halides. Whitmore & Fleming (1933) have shown that β-substituted alkyl halides, especially neopentyl halides, are very inert towards the ordinary nucleophilic reagents. These facts have been interpreted as arising from the large steric hindrance to the approach of attacking ions exerted by these halides (Dostrovsky, Hughes & Ingold, 1946, 1948).

The best results were indeed achieved by substitution of methyl groups in the β-position of the alcohol used for esterification. *iso*Butyl hexenoate was formed in comparatively good yield from hexanoic acid by the usual procedure. Still better results were obtained with neopentyl α-bromohexanoate, the overall conversion of hexanoic acid to the hexenoate ester being 49 %. It was interesting to find that benzyl hexenoate could also be obtained in relatively good yield, 24 % overall. All the comparative dehydrobrominations were carried out in quinoline, the results of which are summarized in Tables 1–3. Further experiments with neopentyl α-bromohexanoate showed that diethylaniline was a slightly more effective dehydrobrominating agent

Table 2. *Yields of esters of α-bromohexanoic acid and of hexenoic acid obtained with α-substituted alcohols*

Ester	Yield of α-bromohexanoate (%)	Yield of hexenoate from hexanoic acid (%)
Methyl	68	0
Ethyl	76	11
*iso*Propyl	63	21
tert.-Butyl	56	6

Table 3. *Yields of esters of α-bromohexanoic acid and of hexenoic acid obtained with β-substituted alcohols*

Ester	Yield of α-bromohexanoate (%)	Yield of hexenoate from hexanoic acid (%)
Ethyl	76	11
n-Propyl	81	19
*iso*Butyl	85	29
Neopentyl	82	49

than quinoline or collidine, the latter being the least useful. Diethylaniline is now used as the reagent of choice as the product obtained is also much cleaner than after the use of quinoline. The material produced by dehydrobromination consists largely of the neopentyl ester of the $\alpha\beta$-unsaturated acid (hex-2-enoic acid), but probably also about 8 % of the $\beta\gamma$ isomer (hex-3-enoic acid; cf. Kon, Linstead & Maclennan, 1932). These isomers may be separated by the method of Fittig (1894), but it was found unnecessary in this work, as saponification, followed by fusion of the mixed acids with potassium hydroxide led only to the formation of butyric and acetic acids. No propionic acid was detected, and the $\beta\gamma$ double bond presumably migrated to the $\alpha\beta$-position in a rearrangement similar to that observed with oleic acid (Varrentrap, 1840; Edmed, 1898). Throughout this paper, the name 'hexenoate' refers to the presumed mixture of the $\alpha\beta$ and $\beta\gamma$ isomers.

The mixture of butyric and acetic acids was purified by steam distillation and separated into its component acids by azeotropic distillation, first with benzene and then with toluene (Schicktanz, Steele & Blaisdell, 1940). It was found that the yields were identical with those obtained from pure hex-2-enoic acid. If the ester were fused before purification or saponification, a rather lower yield was obtained. The analysis of the fatty acid mixtures was carried out by paper chromatography (Brown, 1950) and by liquid-vapour partition chromatography (James & Martin, 1951). In order to obtain a pure specimen of acetic acid, it was found necessary to conduct the azeotropic distillation with benzene very slowly, so that maximum equilibrium might be established in the fractionating column. Analysis by liquid-vapour partition chromatography had shown that approximately equimolar quantities of butyric acid and acetic acid were produced during fusion of hexenoic acid with potassium hydroxide. These results were confirmed in the fractionation, each acid being obtained in 40–45 % yield from hexanoic acid.

The butyric and acetic acids obtained could be further degraded by conventional methods (Calvin, Heidelberger, Reid, Tolbert & Yankwich, 1949). [*Carboxy*-^{14}C]-hexanoic acid was now prepared from *n*-amyl magnesium bromide and [^{14}C]-carbon dioxide. Degradation by the above-described method revealed the presence of radioactivity only in the carboxyl carbon of the acetate in theoretical amount, demonstrating the absence of any rearrangement of the carbon atoms in the course of the series of reactions. A description of the application of this new method for the identification of radioactive atoms in hexanoic acid synthesized from [*carboxy*-^{14}C] acetate in the mammary gland of a lactating goat has already been published (French, Hunter, Martin & Popják, 1951; Popják, French, Hunter & Martin, 1951).

The method has been extended to the degradation of octanoic acid with satisfactory results. Neopentyl α-bromo-octanoate was obtained in 96 % yield from octanoic acid, and the corresponding neopentyl octenoate (presumably a mixture of the $\alpha\beta$ and $\beta\gamma$ isomers) in an overall yield of 59 %. Oct-2-enoic acid had previously been prepared by Bourguel (1929) by catalytic reduction of oct-2-inic acid.

Saponification of neopentyl octenoate and fusion of the potassium salt with a further quantity of potassium hydroxide led to the formation of hexanoic acid in high yield, the conversion of octanoic to hexanoic acid proceeding in 51 % overall yield. The yield of acetic acid by azeotropic distillation was, however, considerably smaller (29 % overall), although analysis of the products of fusion by liquid-vapour partition chromatography showed that equimolar quantities of acetic and hexanoic acids were formed. This further revealed the presence of about 5–10 % of octanoic acid which could be separated from the hexanoic acid by azeotropic distillation with xylene.

EXPERIMENTAL

Fusion of hex-2-enoic acid and methyl hex-2-enoate with potassium hydroxide

After hex-2-enoic acid (0·5 g.) had been heated with solid KOH (1·5 g.) at 250° for 1 hr., paper chromatographic analysis of the product revealed unchanged hexenoic acid only. However, when the fusion of hex-2-enoic acid (3·1 g.) was carried out with KOH (10·0 g.) at 300–340° for 5–10 min., chromatographic analysis by the method of James & Martin (1951) demonstrated the formation of equimolar quantities of butyric and acetic acids only. After fusion, the molten mass was allowed to cool, dissolved in a small volume of water and extracted with ether to remove impurities. The aqueous solution was then made acid and steam-distilled. The mixture of acetic and butyric acids thus obtained was neutralized and the solution evaporated to dryness. The two acids were separated by azeotropic distillation (Schicktanz *et al.* 1940) which gave 1·63 g. (81 %) of acetic acid and 1·99 g. (83 %) of butyric acid. Both were pure chromatographically.

Methyl hex-2-enoate was prepared by treating hex-2-enoic acid (10·0 g.) with a solution of diazomethane (5·6 g.) in ether (500 ml.). The mixture was allowed to stand for 24 hr. when the ether and the residual diazomethane were removed by distillation. The residue was taken up in fresh ether (250 ml.) and washed with two portions of water (150 ml. each). The ethereal solution was dried with anhydrous Na_2SO_4, after which the ether was evaporated off. The crude product was distilled at 62–64°/19 mm. to give methyl hex-2-enoate (8·7 g., 78 %). (Found: C, 65·5; H, 9·4. Calc. for $C_7H_{12}O_2$: C, 65·6; H, 9·4 %.) Some unreacted hex-2-enoic acid could be recovered. This ester had previously been prepared by reacting hex-2-enoic acid with methanol in the presence of H_2SO_4 (Baker, Querry, Safir & Bernstein, 1947).

Fusion of methyl hex-2-enoate with KOH at 300–340° gave butyric and acetic acids in a yield similar to that obtained after the fusion of the free acid.

Experiments with α-bromohexanoic acid

α-Bromohexanoic acid was prepared as described in *Organic Syntheses* (1925). This acid (4·0 g.) was treated with diazomethane (2·8 g.) in ether (200 ml.). The reaction took the normal course, and after standing for 12 hr., the ether and residual diazomethane were evaporated off *in vacuo*. The residue distilled at 90–92°/20 mm. yielding methyl α-bromohexanoate (3·5 g., 81 %). (Found: C, 40·2; H, 6·0; Br, 38·4. $C_7H_{13}O_2Br$ requires C, 40·2; H, 6·2; Br, 38·3 %.)

α-Bromohexanoic acid (0·5 g.) was fused with solid KOH (1·0 g.) at 300–340° for 2 hr. Paper chromatographic analysis of the products revealed the presence of pentanoic (valeric) acid, together with a small amount of butyric and acetic acid.

Methyl α-bromohexanoate (10·0 g.) was refluxed with quinoline (30·0 g.) for a few minutes and allowed to cool. The solution was poured into an excess of 4 N-HCl and then extracted with ether. After drying, the ether was evaporated off leaving a crude brown oil (3·5 g.). Distillation at 92°/25 mm. gave the lactide ester of α-hydroxyhexanoic acid (3·2 g., 54 %) as a colourless oil.

[Carboxy-^{14}C]-*hexanoic acid*

[*Carboxy*-^{14}C]-hexanoic acid was prepared from *n*-amyl bromide and $^{14}CO_2$ by the Grignard reaction, the detailed procedure being similar to that used by Anker (1948) for the preparation of (*carboxy*-^{14}C)-acetic acid.

Preparation of alkyl α-bromohexanoates

These esters were all prepared by similar procedures, of which the preparation of *iso*butyl α-bromohexanoate given below may be taken as an example. Hexanoic acid was heated under reflux with a 50 % excess of thionyl chloride for 1 hr.; unreacted thionyl chloride was removed by distillation. Without further purification, the residual hexanoyl chloride was treated with a 5 % excess of dry bromine, and heated under reflux at about 80° until practically all the free bromine had disappeared. A portion of the crude α-bromohexanoyl chloride (15·0 g.) was poured into *iso*butanol (25·0 g.) and the resulting solution allowed to stand for 12 hr. The excess alcohol was removed *in vacuo*, and the residue was distilled at 125–135°/38 mm., yielding *iso*butyl α-bromohexanoate (15·07 g., 85 %) as a colourless oil with a sickly odour. This product was used as such for further stages in the degradation, but if an analytically pure sample was required, the ester was washed with saturated sodium bicarbonate solution and then with distilled water. Redistillation gave a very pure sample. The physical constants and analytical data for the various α-bromohexanoates are listed in Table 4.

Preparation of alkyl hexenoates

The conversion of the esters of α-bromohexanoic acid to the corresponding esters of hexenoic acid is again typified by the example of the *iso*butyl ester given below. *iso*Butyl α-bromohexanoate (14·09 g.) was dissolved in quinoline (72·0 g.) and heated under reflux. When the temperature reached about 180°, the solution deepened in colour to a dark red and the temperature rose almost instantly to the boiling point. The external source of heat was at once removed, and the solution allowed to cool. The excess quinoline was removed by extraction with a twofold excess of 4 N-HCl and separation of the liquid phases. The aqueous solution was extracted with ether, and the extract added to the previously separated crude *iso*butyl hexenoate. After drying, the ether was evaporated off, and the residual brownish oil distilled at 92–98°/19 mm., yielding *iso*butyl hexenoate (3·25 g., 34 %).

Degradation of neopentyl α-bromohexanoate to butyric and acetic acid

Neopentyl α-bromohexanoate (3·89 g.) was heated under reflux with diethylaniline (6·60 g.) for 6 hr. The solution was cooled and poured into a 100 % excess of 20 % (v/v) aqueous H_2SO_4. Extraction with ether, drying and removal of the solvent left a yellow oil. Distillation at 95–96°/18 mm. gave neopentyl hexenoate (1·65 g., 62 %) as a colourless, sweet-smelling liquid. This ester (1·65 g.) was heated under reflux for 1 hr. with a solution of KOH (1·5 g.) in a mixture of ethanol (10 ml.) and water (5 ml.). The solution was evaporated to dryness on the steam bath and a further quantity of KOH (4·5 g.) added. Fusion at 300–340° was carried out for 10 min., and the molten mass was allowed to cool before extraction with water (30 ml.). The aqueous solution was extracted with ether to remove impurities, and then acidified with H_2SO_4. Steam distillation was then carried out, and the distillate was shown by liquid-vapour partition chromatography to contain equimolar quantities of acetic and butyric acid. The distillate required 15·90 ml. of N-NaOH for neutralization, equivalent to a yield of 89 %, similar to that obtained by fusion of pure hex-2-enoic acid with KOH. Direct alkaline fusion of unsaponified neopentyl hexenoate or of the crude undistilled ester gave lower yields (approx. 75 %).

Degradation of octanoic acid to hexanoic acid

Octanoic acid (10·0 g.) was heated under reflux with thionyl chloride (14 ml.) for 1 hr. The excess of reagent was removed by distillation through a small column at atmo-

Table 4. *Physical constants and analytical data of esters of α-bromohexanoic acid*

			Analysis					
			Found (%)			Requires (%)		
Ester	b.p.	Refractive index	C	H	Br	C	H	Br
Ethyl	120–122°/41 mm.	n_D^{20}1·4509	—	—	—	—	—	—
*iso*Propyl	100–101°/25 mm.	n_D^{20}1·4432	45·4	6·9	34·0	45·6	7·20	33·80
tert.-Butyl	103–104°/18 mm.	n_D^{20}1·4682	48·7	7·7	32·0	47·8	7·60	31·90
n-Propyl	110–111°/20 mm.	n_D^{22}1·4569	45·2	6·7	33·2	45·6	7·15	33·75
n-Capryl	160–161°/18 mm.	n_D^{18}1·4534	54·5	8·7	25·8	54·7	8·80	26·10
*iso*Butyl	115–116°/22 mm.	n_D^{20}1·4356	47·6	7·9	32·1	47·8	7·60	31·90
Neopentyl	120–122°/20 mm.	n_D^{23}1·4530	49·5	7·9	30·5	49·8	7·90	30·20
Benzyl	124–125°/0·4 mm.	n_D^{23}1·5253	—	—	27·5	—	—	28·05

spheric pressure, and bromine (3·8 ml., dried with conc. H_2SO_4) was added to the residue. After heating under reflux at 80–100° for a further 2–3 hr., by which time practically all the free bromine had disappeared, the product was added to a threefold excess of neopentyl alcohol. The dark brown solution obtained was allowed to stand for several hours before removing the excess of neopentanol under reduced pressure. The residue distilled at 148–155°/22 mm., yielding *neopentyl α-bromo-octanoate* (19·53 g., 95 %). Analytically pure material was obtained by washing with saturated aqueous $NaHCO_3$, extraction with ether and drying over anhydrous Na_2SO_4. After removal of the ether, distillation at 89°/0·5 mm. gave the pure ester. (Found: C, 53·1, H, 8·8; Br, 27·0. $C_{13}H_{25}O_2Br$ requires C, 53·2; H, 8·5; Br, 27·3 %.) For degradative purposes the once-distilled material proved satisfactory, as was the case with neopentyl α-bromohexanoate. Neopentyl α-bromo-octanoate (13·58 g.) was treated with diethylaniline (21·0 g.) and heated under reflux for 6 hr. The solution was then only slightly discoloured. It was poured into a large excess of 20 % H_2SO_4 to remove the residual diethylaniline and the resulting aqueous solution was extracted three times with an equal volume of ether. The ethereal extract was dried over anhydrous Na_2SO_4, and the ether then removed. The residue was distilled at 124–127°/19 mm., giving *neopentyl octenoate* (6·05 g., 62 %) as a colourless, sweet-smelling oil. The ester was further purified with only trivial loss by redistillation at 118–120°/18 mm. (Found: C, 74·0; H, 11·4. $C_{13}H_{24}O_2$ requires C, 73·6; H, 11·3 %.)

Neopentyl octenoate (0·48 g.) was dissolved in ethanol (3 ml.) and a solution of KOH (0·5 g.) in water (1·5 ml.) added. The mixture was heated under reflux for 2 hr. and evaporated to dryness on the steam bath. A further quantity of KOH (2·0 g.) was added, and fusion was then carried out at 300–350° until gas evolution practically ceased. The product was dissolved in water (30 ml.) and non-acidic impurities removed by ether extraction. The aqueous solution was now neutralized with H_2SO_4. Vapour phase chromatography revealed the presence of equivalent amounts of acetic and hexanoic acid, but also a smaller quantity (5–10 % of the hexanoic acid) of octanoic acid. The fusion was repeated under the same conditions with a further portion of neopentyl octenoate (1·00 g.), but this time the acidified aqueous extract was steam distilled. The mixed acids in the steam distillate were neutralized, and the water then evaporated off. The free acids were liberated from the salts by treatment with a large excess of *p*-toluenesulphonic acid in dry benzene, and fractionated by azeotropic distillation, first with benzene, and then with xylene, any octanoic acid remaining behind. Chromatographically pure acetic acid (0·131 g., 46 %) and hexanoic acid (0·465 g., 85 %) were obtained. Thus the overall conversion of octanoic to hexanoic acid was 51 %.

SUMMARY

1. A new method for the degradation of *n*-carboxylic acids is described in which two carbon atoms are removed from the carboxyl end as acetic acid, the other product being a *n*-carboxylic acid with two carbon atoms less than the original acid.

2. The chemical reactions involved in the degradation are: (i) conversion of the carboxylic acid to the corresponding acid chloride; (ii) bromination of the acid chloride; (iii) conversion of the α-bromo acid chloride to an ester by reaction with an alcohol; (iv) dehydrobromination of the resulting α-bromo ester; and (v) saponification of the unsaturated ester produced in reaction (iv), and fusion of the salt with solid potassium hydroxide.

3. Hexanoic acid was used in model experiments, and wide variation was found in the yields obtained in the dehydrobromination stage when different esters were used. The neopentyl ester gave the best yield.

4. Diethylaniline was found to be a more satisfactory reagent for the dehydrobromination of the α-bromo esters than either quinoline or collidine.

5. [*Carboxy*-^{14}C]-hexanoic acid has been synthesized, and the degradation carried out on this labelled material. Radioactive carbon was found only in the carboxyl carbon of the acetic acid obtained by the degradation indicating that rearrangement of the carbon atoms did not occur during the reactions.

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Isolation of Antibiotics from a Species of *Cephalosporium*. Cephalosporins P_1, P_2, P_3, P_4 and P_5

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Brotzu (1948) reported that a species of *Cephalosporium* isolated from the sea near Sardinia produced a substance that inhibited the growth of a number of Gram-positive and Gram-negative bacteria. The production of antibacterial substances by the same *Cephalosporium* has recently been studied in this laboratory and in the Antibiotics Research Station of the Medical Research Council at Clevedon (Crawford, Heatley, Boyd, Hale, Kelly, Miller & Smith, 1951). This paper describes the isolation and some of the properties of members of one group of antibiotics obtained from active culture filtrates of the organism.

At an early stage of the present work it was found that some culture fluids contained at least two kinds of antibacterial substance, one of which could readily be extracted into butyl acetate from a weakly acid solution while the other was insoluble in common organic solvents. The active material in the ester-soluble fraction, with which this paper is concerned, was called cephalosporin P. The remaining water-soluble substance, which was discovered independently at Clevedon, had quite different chemical and antibacterial properties. It was named cephalosporin N and will be the subject of a separate publication. Solutions of cephalosporin P inhibit the growth of *Staphylococcus aureus* but not of *Salmonella typhi*, whereas cephalosporin N is active against both organisms. It is probable that the crude extracts prepared by Brotzu (1948) contained mainly cephalosporin N, while the material studied in earlier work at Oxford (Crawford *et al.* 1951), which could be extracted by organic solvents, was cephalosporin P.

It has now been shown that crude cephalosporin P contains five acidic antibiotics. These substances have been named cephalosporins P_1, P_2, P_3, P_4 and P_5. Cephalosporins P_1, P_2 and P_4 have been isolated in crystalline form. All five substances have been found to have some properties in common with helvolic acid, an antibiotic produced by various strains of *Aspergillus fumigatus* (Chain, Florey, Jennings & Williams, 1943).

Ritchie, Smith & Florey (1951) have shown that cephalosporin P_1 is almost as active as aureomycin and terramycin against the staphylococcus *in vitro*, and that it is less toxic than these substances when given intravenously and can readily be obtained in the blood in bacteriostatic concentration. For reasons that are not yet understood, however, cephalosporin P_1 has proved much less effective than aureomycin or terramycin in curing staphylococcal infections in mice.

Isolation of cephalosporins P_1, P_2 and P_4

Culture fluids obtained from the Antibiotics Research Station at Clevedon contained much larger amounts of cephalosporin P_1 than of other components of the P complex, and the process of purification was directed towards the isolation of this substance. The minor active constituents occurred in variable amounts in different batches of material. Information concerning cephalosporins P_2, P_3, P_4 and P_5 was accumulated in the course of the work, during which the various fractions obtained were examined by paper chromatography. The cylinder-plate method of assay (Heatley, 1944), with *Staph. aureus* as a test organism, was used to follow the active substances.

The acidic cephalosporin P could be extracted almost completely from an aqueous solution at pH 6·5 into one-fifth of a volume of butyl acetate, and since the extract could be concentrated by distillation without loss of activity this procedure was used for removing the antibiotics from large