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Cephalosporin P₁ and Helvolic Acid

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The isolation of antibiotics from the culture fluid of a species of *Cephalosporium* was described by Burton & Abraham (1951). These substances, which were called cephalosporin P₁, P₂, P₃, P₄ and P₅, appeared to belong to the same chemical family. They resembled helvolic acid, an antibiotic produced by *Aspergillus fumigatus* mut. *helvola* Yuill (Chain, Florey, Jennings & Williams, 1943) in some of their chemical and biological properties. All the antibiotics were inactivated by an enzyme produced by certain strains of aerobic spore-forming organisms. A strain of *Staphylococcus aureus* which had become resistant to cephalosporin P₁ was found to be resistant to helvolic acid and vice versa.

Of the members of the cephalosporin P family, only cephalosporin P₁ was obtained in a quantity sufficient for detailed chemical study. This paper records some observations on the nature of cephalosporin P₁ (subsequently referred to as cephalosporin), and its relationship to helvolic acid. Cephalosporin (m.p. 147°; $[\alpha]_D^{20} + 28^\circ$) is an acid which contains carbon, hydrogen and oxygen only. The results of elementary analysis were difficult to

interpret until it was realized that the antibiotic crystallized from hydroxylic solvents with solvent of crystallization, but the empirical formula C₃₂H₄₈O₈ now appears to be firmly established. The molecular weight of material crystallized from aqueous ethanol (dried *in vacuo* over P₂O₅), determined by X-ray diffraction measurements by Miss M. Curzon and Dr Dorothy Hodgkin, was 601 ± 16. This value, and the analytical figures, are in excellent agreement with the molecular formula C₃₂H₄₈O₈·H₂O, $\frac{1}{2}$ C₂H₅OH.

Cephalosporin, like helvolic acid, is a monobasic carboxylic acid. Electrometric titration in 60% (v/v) ethanol showed the presence of a group with a pK of 6.8 (equivalent weight 610). This would correspond to a pK of approximately 4.9 in water, i.e. to that expected of an aliphatic carboxyl group not linked closely to a strongly electronegative centre. The infrared spectrum of cephalosporin showed a broad band at 5.77–5.86 μ. due to the presence of carbonyl groups. In the potassium salt a new band, assigned to the –CO₂–group, appeared at 6.45 μ., but a broad band at 5.8 μ. still remained.

On methylation with diazomethane at 0° cephalosporin forms a non-acidic monomethyl ester, C₃₃H₅₀O₈ (m.p. 196°; $[\alpha]_D^{10} + 28^\circ$). This ester, like methyl helvolate, melts some 50° higher than

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the parent acid. Its molecular weight was found by X-ray diffraction measurements to be 572 ± 16 . Methylation of cephalosporin with diazomethane at room temperature leads to the formation of material of lower melting point containing nitrogen.

Both cephalosporin and its methyl ester show strong absorption at 211–218 $m\mu$. ($\epsilon_{\max}$, approx. 10 000), but no other band in the ultraviolet region. The absence of absorption of low extinction above 240 $m\mu$. indicates that keto groups are not present, and in this respect cephalosporin differs from helvolic acid, which has two keto groups. In

rapidly lactonized on acidification to give a neutral substance of the composition $C_{28}H_{42}O_8$ (m.p. 186° ; $[\alpha]_D^{20} + 56.5$). Acetyl determinations on these substances indicated that two acetate groups had been successively hydrolysed, and this was confirmed by titration of the steam-volatile acid formed on hydrolysis and its identification as acetic acid by paper chromatography (Hiscox & Berridge, 1950; Burton, 1954). The first substance is therefore monodesacetylcephalosporin and the second bisdesacetylcephalosporin lactone, the reactions being as shown below:

contrast to helvolic acid, cephalosporin does not react with the Girard reagent (Girard & Sandulesco, 1936) and shows a negative Zimmermann reaction (Fieser & Fieser, 1949).

Cephalosporin and its methyl ester rapidly absorb one molecular equivalent of hydrogen in the presence of Adams platinum oxide catalyst, yielding dihydrocephalosporin and dihydrocephalosporin methyl ester ($C_{35}H_{52}O_8$) respectively. The dihydro ester, unlike the parent ester, gives no colour with tetranitromethane (Ruzicka, Huyser, Pfeiffer & Seidel, 1929), but still shows selective absorption in the ultraviolet region ($\lambda_{\max}$, 222 $m\mu$; $\epsilon_{\max}$, 6900). Hence, a non-conjugated double bond has been reduced. Further hydrogenation in acetic acid containing hydrochloric acid proceeded slowly with the formation of a variety of products whose nature became apparent only after the behaviour of cephalosporin on hydrolysis had been studied. However, the products had absorbed at least two more hydrogen atoms and showed no selective absorption in the ultraviolet region. It was concluded that cephalosporin contained one isolated double bond which was readily reduced and a double bond conjugated with the carboxyl group. The second double bond is probably fully substituted, as no band in the region 6.0–6.5 μ . was visible in the infrared spectrum of suitable derivatives. A model mixture of

and its $\beta\gamma$ -isomeride (Jocelyn & Polgar, 1953) was similar in this respect.

On standing in normal aqueous sodium hydroxide at 37° cephalosporin slowly deposited a crystalline sodium salt. The acid derived from this salt (m.p. 220° ; $[\alpha]_D^{20} + 32.5^\circ$) had the composition $C_{30}H_{46}O_7$, suggesting that it was formed by the loss of an acetyl group from the parent compound. Further hydrolysis with alkali yielded a product which

In a similar manner, mild alkaline hydrolysis of cephalosporin methyl ester gives the methyl ester of monodesacetylcephalosporin (m.p. 241°), which is also formed by methylation of monodesacetylcephalosporin with diazomethane. Further alkaline hydrolysis of monodesacetylcephalosporin methyl ester, and acidification of the product, give the bisdesacetyl lactone. The hydrolysis of dihydrocephalosporin methyl ester follows a similar path.

The elucidation of the two-stage hydrolysis of cephalosporin allowed an interpretation of similar experiments on the hydrolysis of helvolic acid by Williams (1952). Helvolic acid ($C_{38}H_{42-44}O_8$) also contains two acetyl groups. One of these groups is removed by mild alkaline hydrolysis, giving a monodesacetylhelvolic acid ($C_{36}H_{40-42}O_7$) and the other by more vigorous hydrolysis, leading to bisdesacetylhelvolic lactone ($C_{28}H_{36-38}O_6$). In the latter compound two oxygen atoms are present in the lactone grouping and one in the hydroxyl group liberated on hydrolysis of the first acetoxy group. The two remaining oxygen atoms appear to be present in keto groups, as helvolic acid formed a derivative with the composition of a dioxime, although it yielded only a monosemicarbazone (Williams, 1952).

The ultraviolet absorption curve of methyl helvolate showed selective absorption at 218 $m\mu$. ($\epsilon_{\max} = 14\,750$) and at 232 $m\mu$. ($\epsilon_{\max} = 16\,150$). The first band was assigned to an $\alpha\beta$ -unsaturated carboxyl group, as in cephalosporin, and the second to an $\alpha\beta$ -unsaturated ketone group. It was therefore conceivable that the so-called dioxime was an hydroxylamino oxime formed by the addition of a second molecule of hydroxylamine to the conjugated double bond. However, this possibility was discounted by the analytical evidence and by the fact that the absorption of the dioxime in the ultraviolet region was very similar to that of helvolic acid. The ultraviolet absorption spectrum

of helvolic acid monosemicarbazone ($\lambda_{\max}$. 214 m μ ., $\epsilon_{\max}$. 12 300; $\lambda_{\max}$. 280 m μ .; $\epsilon_{\max}$. 12 200) showed that the $\alpha\beta$ -unsaturated carbonyl group was involved in the reaction with semicarbazide.

In addition to the postulated $\alpha\beta$ -unsaturated ketone group helvolic acid contains a further easily reducible double bond. Under conditions which lead to the formation of a dihydro derivative from cephalosporin methyl ester, methyl helvolate absorbed two molecular equivalents of hydrogen, yielding methyl tetrahydrohelvolate. The ultraviolet absorption spectrum of methyl tetrahydrohelvolate ($\lambda_{\max}$. 216 m μ ., $\epsilon_{\max}$. 13 000) indicated that it still contained an $\alpha\beta$ -unsaturated ester grouping.

The remaining two oxygen atoms in cephalosporin are not present as keto groups, and one at least is present as a hydroxyl group, since the infrared spectrum of cephalosporin methyl ester shows a strong OH band at 3 μ . Acetylation of cephalosporin methyl ester under a variety of conditions did not lead to crystalline derivatives, but analysis of the products indicated that two acetyl groups could be introduced into the molecule. It is therefore probable that both the remaining oxygen atoms in cephalosporin belong to hydroxyl groups.

Oxidation of cephalosporin and its methyl ester with chromic acid in acetone led to ketonic products whose carbon content was lower than that required for the diketo derivatives, but oxidation of dihydrocephalosporin methyl ester gave a compound (m.p. 111–113°; $[\alpha]_D^{20} + 70.7^\circ$) whose composition was that of the expected methyl ester of bisdehydrodihydrocephalosporin (C₃₃H₄₈O₈). Oxidation of bisdesacetylcephalosporin lactone yielded a yellow product with the composition C₂₈H₃₆O₆, which was presumably a triketo lactone, since its infrared spectrum showed the absence of hydroxyl groups.

Hydrogenation of the conjugated double bond in dihydrocephalosporin methyl ester, which could be accomplished with Adams platinum oxide catalyst in acetic acid containing a small amount of concentrated hydrochloric acid, was accompanied by the removal of acetyl groups from the molecule. The product was a mixture whose components were not isolated in crystalline form; but among the fractions obtained by chromatography on alumina was one with the composition of monodesacetyl-tetrahydrocephalosporin methyl ester (C₃₁H₅₂O₇) and another with the composition of bisdesacetyl-tetrahydrocephalosporin methyl ester (C₂₉H₅₀O₈). During the reduction more hydrogen was, however, absorbed than that required to saturate one double bond. Presumably some hydrogenolysis occurred in addition to hydrolysis. These complications were avoided in the complete hydrogenation of

bisdesacetylcephalosporin lactone. The latter compound absorbed two molecular equivalents of hydrogen to yield a crystalline tetrahydro lactone (C₂₈H₄₆O₆; m.p. 235°; $[\alpha]_D^{20} + 27^\circ$).

This work, therefore, leads to the conclusion that cephalosporin is

and that helvolic acid is

The C₂₇H₃₉ fragment of cephalosporin contains two double bonds and the C₂₇H₃₅ fragment of helvolic acid contains three double bonds. Both compounds are therefore tetracyclic. The precise relationship of the two substances, however, remains uncertain, for the compound obtained by oxidation of dihydrocephalosporin methyl ester, which was thought to be bisdehydrodihydrocephalosporin methyl ester, proved to be quite different from methyl tetrahydrohelvolate. Possibly the keto groups in these two compounds are in different positions, or the reduction of the extra double bond in helvolic acid leads to a different stereochemical series.

Since cephalosporin and helvolic acid appear to be tetracyclic compounds with a C₂₈ skeleton, the question arises whether they are steroids. A steroid structure would be consistent with the fact that both compounds give positive Liebermann–Burchard and reversed Salkowski reactions, and that helvolic acid and the keto derivatives of cephalosporin give a positive Zimmermann reaction (Fieser & Fieser, 1949). These colour reactions, however, are not specific for compounds of the steroid series, but are given by many other unsaturated polynuclear hydroaromatic substances. Before the structure of the carbon skeleton of cephalosporin and helvolic acid can be established it will be necessary to characterize the products of more extensive degradations than those described in this paper.

The antibacterial activity of cephalosporin (Burton & Abraham, 1951) is not dependent on the presence of the easily hydrogenated double bond, for dihydrocephalosporin is as active as the antibiotic itself. Changes in other parts of the molecule, however, are accompanied by complete or

* An examination of the published analytical figures (Williams, 1952) suggests that the formula for helvolic acid is C₃₂H₄₂O₈ rather than C₃₂H₄₄O₈.

substantial loss of activity. Prolonged hydrogenation of dihydrocephalosporin in ethyl acetate, which presumably brings about the reduction of the $\alpha\beta$ -unsaturated carboxylic acid group, leads to an amorphous product which is inactive against *Staph. aureus*. Monodesacetylcephalosporin shows about one-eighth of the activity of cephalosporin itself when tested on plates seeded with *Staph. aureus*, and monodesacetylhelvolic acid shows a similar decrease of activity in comparison with helvolic acid. The chromic acid oxidation products of cephalosporin are inactive. The high antibacterial activity of cephalosporin, like that of many other antibiotics, appears to be a consequence of the combined effect of several different features of the molecule.

EXPERIMENTAL

Ultraviolet and infrared spectra. These were measured by Dr F. B. Strauss with the technical assistance of Mr F. H. L. Hastings. Ultraviolet absorption spectra were measured in methanol, with a Beckman or Unicam spectrophotometer. Infrared spectra were measured in paraffin paste, with a Perkin-Elmer Double Beam Photometer, Model 21.

Cephalosporin. The antibiotic separates from 50% (v/v) aqueous ethanol in orthorhombic crystals, m.p. 147°, $[\alpha]_D^{20} + 28^\circ$ in chloroform (c, 2.7), $+28^\circ$ in chloroform (c=1.0). [Found: C, 66.0, 66.0; H, 9.1, 9.1. (Dried for 3 hr. at 100° and at 0.05 mm.) $C_{33}H_{48}O_8 \cdot \frac{1}{2}C_2H_5OH$ requires C, 66.3; H, 8.9%.] Miss M. Curzon and Dr D. Hodgkin, of the Department of Crystallography, Oxford, provided the following report on the crystals: space group $P2_12_12_1$ or $P2_12_12_2$. Cell dimensions: a, 6.95 Å; b, 21.03 Å; c, 23.7 Å; d, obtained by flotation in a solution of $KHgI_3$, 1.152. Assuming 4 mol. in the unit cell, the mol.wt. was calculated to be 601 ± 16 . The mol.wt. of $C_{33}H_{48}O_8 \cdot \frac{1}{2}C_2H_5OH$ is 598. The ultraviolet absorption spectrum of cephalosporin showed λ_{max} 211 m μ , ϵ_{max} 9140.

Cephalosporin is readily soluble in chloroform and in ethanol, but sparingly soluble in hexane and in water. The solubility of the sodium salt in water is about 10 mg./ml. Solutions of cephalosporin in 95% (v/v) ethanol gave no ppt. with digitonin.

Cephalosporin gave a yellow colour with tetranitromethane in chloroform. It gave no colour with $FeCl_3$ and did not reduce Tollens' reagent. The Legal, Rosenheim and Tortelli-Jaffé tests (Fieser & Fieser, 1949) were negative.

Cephalosporin methyl ester. Cephalosporin (500 mg.) was dissolved in ether (30 ml.), the solution cooled to 0° and an ethereal solution of diazomethane added until the yellow colour was no longer discharged (1 equiv.). After 30 min. the solution was washed successively with 0.1N-HCl, $NaHCO_3$ solution, and water. It was then dried over anhydrous Na_2SO_4 and the ether removed by distillation. The solid residue was crystallized from aqueous methanol and yielded 495 mg. of methyl ester. *Cephalosporin methyl ester* had m.p. 196°; $[\alpha]_D^{20} + 27^\circ$ in chloroform (c, 2.4), $+28^\circ$ in chloroform (c, 1). (Found: C, 68.9, 68.9; H, 8.9, 8.8; acetyl, 17.0; OMe, 4.6. $C_{33}H_{50}O_8$ requires C, 68.9; H, 8.7%; 2 acetyl 15.0%; 1 OMe requires 5.4%.) Miss M. Curzon and Dr D. Hodgkin reported on the crystals as follows:

orthorhombic; space group $P2_12_12_1$ or $P2_12_12_2$. Cell dimensions: a, 7.14 Å; b, 19.54 Å; c, 23.92 Å; d, by flotation in a solution of $KHgI_3$, 1.138. Assuming 4 mol. in the unit cell, the mol.wt. was calculated to be 572 ± 16 . The mol.wt. required for $C_{33}H_{50}O_8$ is 574.

The ultraviolet absorption spectrum of cephalosporin methyl ester showed λ_{max} 213 m μ , ϵ_{max} 11650; λ_{max} 218 m μ , ϵ_{max} 11570.

The compound gave a yellow colour with tetranitromethane in chloroform. It formed no ppt. with digitonin in 95% (v/v) ethanol.

Dihydrocephalosporin. Cephalosporin (100 mg.) was dissolved in 1 equiv. of NaOH and the aqueous solution (6 ml., pH 7.2) shaken with Adams platinum oxide catalyst (10 mg.) under H_2 . After 3 hr., when slightly more than 1 mol.prop. of H_2 had been absorbed, the solution was filtered from the catalyst, adjusted to pH 3 with 0.1N-HCl, and extracted three times with half its volume of ether. The ethereal solution was dried over anhydrous Na_2SO_4 and evaporated, to yield a white solid which gave a negative Liebermann-Burchard reaction. After crystallization from aqueous methanol this yielded *dihydrocephalosporin*, which had m.p. 155°; $[\alpha]_D^{20} + 46^\circ$ in chloroform (c, 0.55). (Found: C, 65.5, 65.5; H, 9.4, 9.3; active H, 0.82%. $C_{33}H_{50}O_8 \cdot \frac{1}{2}C_2H_5OH$ requires C, 65.6; H, 9.2; 4.5 active H, 0.72%.)

Dihydrocephalosporin methyl ester. Cephalosporin methyl ester (100 mg.) was dissolved in ethyl acetate (10 ml.) and shaken under H_2 with Adams catalyst (20 mg.). 1 mol. prop. of H_2 was absorbed in 12 min. The solution was filtered from the catalyst and evaporated to dryness. The residue was crystallized from aqueous methanol, yielding *dihydrocephalosporin methyl ester*, m.p. 214°. The same compound was obtained by methylating dihydrocephalosporin with diazomethane in ether. (Found: C, 68.5, 68.6; H, 9.0, 9.1. $C_{33}H_{52}O_8$ requires C, 68.7; H, 9.1%.)

Dihydrocephalosporin methyl ester gave no colour with tetranitromethane. Its ultraviolet absorption spectrum showed λ_{max} 222 m μ ; ϵ_{max} 6900.

Monodesacetylcephalosporin. Cephalosporin (100 mg.) was dissolved in an excess (5 ml.) of aqueous N-NaOH and the solution kept at room temp. for 30 min. The solution was acidified carefully with 11N-HCl and the resulting ppt. was crystallized from methanol, giving *monodesacetylcephalosporin* in nearly theoretical yield. This compound had m.p. 220°; $[\alpha]_D^{20} + 32.5^\circ$ in methanol (c, 1.5). (Found: C, 69.4, 69.6; H, 8.9, 8.9; acetyl 9.0%; mol.wt. (Rast) 550. $C_{30}H_{46}O_7$ requires C, 69.4; H, 8.9; acetyl 8.4%; mol.wt. 518.)

Monodesacetylcephalosporin was also formed when cephalosporin sodium salt was kept in 1 equiv. of 0.1N-NaOH solution at 37° for 3 days, or when cephalosporin was heated under reflux for 1 hr. with 2 equiv. of ethanolic 0.2N-KOH.

Monodesacetylcephalosporin is less soluble in methanol than cephalosporin and its sodium salt is less soluble in water than the sodium salt of cephalosporin. On further hydrolysis with aqueous NaOH it yields a product which forms bisdesacetylcephalosporin lactone on acidification.

Monodesacetylcephalosporin methyl ester. Cephalosporin methyl ester (152 mg.) was dissolved in ethanol (13 ml.) containing 1 equiv. of NaOH and the solution was kept at 80° for 30 min. The solution was then reduced to 2 ml. and diluted with water (5 ml.). The ester separated and was crystallized from methanol. It had m.p. 241; $[\alpha]_D^{20} + 28.7^\circ$ in

chloroform (c, 1.1). (Found: C, 69.9; H, 9.0; OMe, 6.9. C₃₃H₄₈O₇ requires C, 69.9; H, 9.0; OMe, 6.1%.)

On further hydrolysis with hot N-NaOH monodesacetylcephalosporin methyl ester neutralized 2 equiv. of alkali and formed a product which yielded bisdesacetylcephalosporin lactone on acidification.

Monodesacetyldihydrocephalosporin methyl ester. Dihydrocephalosporin methyl ester (50 mg.) was treated with 1 equiv. of 0.3N-NaOH and the mixture left at 37° for 4 days. The insoluble ester was removed by filtration and crystallized from aqueous methanol. It melted at 261° and gave no colour with tetranitromethane in chloroform. (Found: C, 69.7; H, 9.7; OMe, 5.3. C₃₁H₅₀O₇ requires C, 69.6; H, 9.5; OMe, 5.8%.) The ultraviolet absorption spectrum showed $\lambda_{\max}$. 219 m μ ., $\epsilon_{\max}$. 8900; $\lambda_{\max}$. 223 m μ ., $\epsilon_{\max}$. 8800.

Bisdesacetylcephalosporin lactone. Cephalosporin (100 mg.) was refluxed for 4 hr. with 8.16 ml. of 0.1N-NaOH. Back titration with HCl showed that 5.15 ml. of 0.1N-NaOH (3 equiv.) had been neutralized. The solution was acidified and the resulting ppt. filtered off, washed with water and crystallized from aqueous methanol or benzene. The lactone had m.p. 186°; $[\alpha]_D^{20} + 56.5^\circ$ in ethanol (c, 1.4). (Found: C, 73.5; H, 9.2; OMe, 9.5. C₂₈H₄₂O₅ requires C, 73.3; H, 9.2%.)

Impure samples of bisdesacetylcephalosporin lactone could be purified by chromatography of a solution in acetone, using acid-washed alumina or Florisil (magnesium silicate, kindly provided by the Floridin Co., Warren, Pa., U.S.A.), and eluting with acetone-ether (1:4 by vol.).

Bisdesacetyldihydrocephalosporin lactone. (a) Dihydrocephalosporin methyl ester (50 mg.) was dissolved in a mixture of 0.5 ml. (3 equiv.) of 0.5N-ethanolic NaOH and 1.5 ml. of ethanol. The solution was kept at 80° for 4 hr. The lactone separated on adding 2 ml. of water and acidifying the solution. It crystallized from aqueous methanol in needles, which had m.p. 222°; $[\alpha]_D^{20} + 71.5^\circ$ in ethanol (c, 1.4). (Found: C, 73.0; H, 9.9. C₂₈H₄₄O₅ requires C, 73.1; H, 9.6%.)

(b) Bisdesacetylcephalosporin lactone (158 mg.) was dissolved in ethyl acetate (10 ml.) and shaken under H₂ with 30 mg. of Adams catalyst. 1 Mol. prop. of H₂ was absorbed in 40 min. The solution was filtered from the catalyst and evaporated to dryness. The product was crystallized from aqueous methanol. It had m.p. 220–222°; $[\alpha]_D^{20} + 71.5^\circ$ in ethanol (c, 0.6), $[\alpha]_D^{20} + 83$ in chloroform (c, 1.8). (Found: C, 72.7; H, 9.6. C₂₈H₄₄O₅ requires C, 73.1; H, 9.6%.)

Bisdesacetyldihydrocephalosporin lactone gave no colour with tetranitromethane in chloroform, but gave a reddish purple colour with the Zimmermann reagent.

Methyl tetrahydrohelvolate. Methyl helvolate (46 mg.) was dissolved in ethyl acetate (10 ml.) and shaken with 20 mg. of Adams catalyst under H₂. 2 mol. prop. of H₂ were absorbed in 50 min. The solution was filtered from the catalyst and evaporated to dryness. The residue was crystallized from aqueous methanol, giving *methyl tetrahydrohelvolate*, m.p. 212°; $[\alpha]_D^{20} - 70^\circ$ in chloroform. (Found: C, 69.9; H, 8.5. C₃₃H₄₈O₈ requires C, 69.2; H, 8.5%.)

This compound showed a positive Zimmermann reaction, but gave no colour with tetranitromethane in chloroform. Its ultraviolet absorption spectrum showed: $\lambda_{\max}$. 216 m μ .; $\epsilon_{\max}$. 13 000.

Bisdehydrodihydrocephalosporin methyl ester. Dihydrocephalosporin methyl ester (500 mg.) was dissolved in acetone (2 ml.) and the solution mixed with a solution of

CrO₃ (400 mg.) in 8N-H₂SO₄ (3 ml.) at 0°. After 30 min. the product was extracted into ether and the ethereal solution washed successively with NaHCO₃ solution and with water. The ether was then removed *in vacuo* and the residue, on crystallization from aqueous methanol, gave a compound which had m.p. 111–113°, $[\alpha]_D^{20} + 70.7^\circ$ in chloroform (c, 0.34). (Found: C, 69.0; H, 8.7; acetyl, 17.9. C₃₃H₄₈O₈ requires C, 69.2; H, 8.5; 2 acetyl, 15.1%.)

Oxidation product of bisdesacetylcephalosporin lactone. Bisdesacetylcephalosporin lactone was oxidized with CrO₃ under the conditions used for the oxidation of dihydrocephalosporin methyl ester. The product was extracted into ether and the ethereal solution washed with NaHCO₃ solution and with water. Evaporation of the ether *in vacuo* left a yellow solid, which had m.p. 160–168°. (Found: C, 74.0; H, 8.1. C₂₈H₃₆O₅ requires C, 74.3; H, 8.0%.)

Further hydrogenation of dihydrocephalosporin methyl ester. Dihydrocephalosporin methyl ester (100 mg.) was dissolved in acetic acid (10 ml.) containing one drop of 11N-HCl. The solution was shaken under H₂ with 50 mg. of Adams catalyst. 2 mol. prop. of H₂ were absorbed in 5 hr. The solution was filtered from the catalyst and evaporated to dryness *in vacuo*. The product, which proved to be a mixture, was soluble in light petroleum (b.p. 60–80°). It was chromatographed on alumina, developing first with light petroleum (b.p. 60–80°), then with a mixture of light petroleum (b.p. 60–80°) and benzene, and finally with a mixture of benzene and ether. The main bulk of the material was eluted with the benzene-ether mixture, and analysed for *monodesacetyltetrahydrocephalosporin methyl ester*. (Found: C, 69.1; H, 9.6; OMe, 8.7; acetyl, 9.9. C₃₁H₅₂O₇ requires C, 69.4; H, 9.8; OMe 5.8; acetyl 8.1%.)

Bisdesacetyltetrahydrocephalosporin lactone. Cephalosporin lactone (94 mg.) was dissolved in glacial acetic acid (10 ml.) containing one drop of 11N-HCl. The solution was shaken under H₂ with 50 mg. of Adams catalyst. 2 mol. prop. of H₂ were absorbed in 5 hr. The solution was filtered from the catalyst and evaporated to dryness *in vacuo*. The *tetrahydro*lactone was crystallized from hot methanol, m.p. 235°; $[\alpha]_D^{20} + 27^\circ$ in methanol (c, 1.7). (Found: C, 72.9; H, 10.4. C₂₈H₄₆O₅ requires C, 72.7; H, 10.0%.)

SUMMARY

1. Cephalosporin P₁, an antibiotic produced by a strain of *Cephalosporium*, is a monocarboxylic acid with the molecular formula C₃₂H₄₈O₈. It is similar to the antibiotic helvolic acid (C₃₂H_{42–44}O₈) in its antibacterial activity and some of its chemical properties.

2. Cephalosporin P₁ contains two acetyl groups and two hydroxyl groups. Helvolic acid contains two acetyl groups and two keto groups. Mild alkaline hydrolysis of cephalosporin P₁ results in the formation of monodesacetylcephalosporin P₁, C₃₀H₄₆O₇. More vigorous hydrolysis removes the second acetyl group and yields a product which forms bisdesacetylcephalosporin P₁ lactone, C₂₈H₄₂O₅, on acidification. Helvolic acid behaves similarly under the same conditions.

3. Cephalosporin P₁, its methyl ester and bisdesacetylcephalosporin P₁ lactone are readily converted into dihydro derivatives on catalytic

hydrogenation. Cephalosporin P_1 also contains a second double bond which is more difficult to reduce. Ultraviolet absorption spectra indicate that the second double bond is part of an $\alpha\beta$ -unsaturated carboxylic acid group. Helvolic acid appears to contain an $\alpha\beta$ -unsaturated carboxylic acid group, an $\alpha\beta$ -unsaturated ketone group and a further easily reducible double bond.

4. It is concluded that cephalosporin P_1 and helvolic acid are tetracyclic compounds with a C_{28} skeleton. Both compounds give colours in the Liebermann-Burchard and Salkowski tests, and helvolic acid shows a positive Zimmermann reaction.

5. Dihydrocephalosporin P_1 shows the same activity against *Staph. aureus* as the antibiotic itself, but the product of further hydrogenation is inactive. Monodesacetylcephalosporin P_1 is about one-eighth as active as cephalosporin P_1 .

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