Rearrangement of penicillins to anhydropenicillins[1]

SAUL WOLFE,[2] J. C. GODFREY, C. T. HOLDREGE, AND Y. G. PERRON[3]
Organic Research Division, Bristol Laboratories Division of Bristol-Myers Company, Syracuse 1, New York

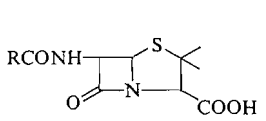
Received October 25, 1967

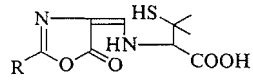
Anhydropenicillins are derived from penicillins by the removal of a molecule of water from the nucleus with concomitant rearrangement of the thiazolidine ring to an α,β-unsaturated-γ-thiolactone. Conditions are described for the preparation of the acid chlorides of penicillins; these compounds rearrange to anhydropenicillins upon treatment with a tertiary amine. Other less effective ways of performing the rearrangement are also given. The anhydropenicillins show infrared absorption at 5.5, 5.9, 6.0, and 6.1 μ, ultraviolet absorption near 270 mμ (ε = 12 000), and they possess extraordinary chemical stability. Possible reasons for these properties are discussed.

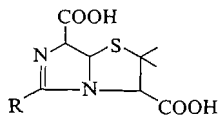
Canadian Journal of Chemistry, 46, 2549 (1968)

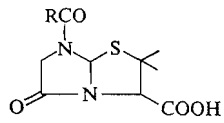
The great variety of the rearrangements that have been reported for penicillins (**1**) is a feature of the chemistry of these compounds which continues to fascinate organic chemists. Until recently, however, all of the known rearrangement products were the result of thermal or acid-catalyzed intramolecular attack on the carbonyl group of the labile β-lactam (for examples see ref. 1). These products include the penicillenic acids (**2**), the penillic acids (**3**), the penillonic acids (**4**), and azlactones (**5**).

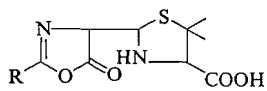
The discovery of the important therapeutic properties associated with *N*-acyl derivatives (**6**) of 7-aminocephalosporanic acid (**7**) (2, 3) has led

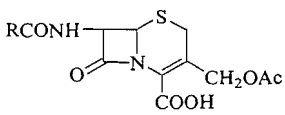

1

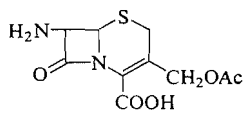

2

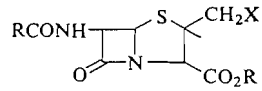

3


4


5


6


7


8

us as well as others to study the possibility of effecting a rearrangement of the *thiazolidine* portion of penicillin without loss of the β-lactam. One objective of this work is the partial synthesis of a cephalosporin (**6**)[4] from a penicillin. Especially valuable for such a transformation would be a compound such as **8** where a hydrogen on one of the methyl groups of penicillin has been replaced by a potential leaving group such as tosyl, halogen, or N_2^+. We expect that such a compound might be induced to rearrange to a cephalosporin derivative by a route such as that shown in Chart 1.[5]

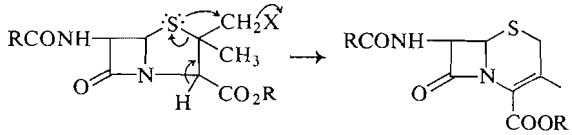


Chart 1

[1]Presented at the XIXth International Congress of Pure and Applied Chemistry, London, 1963, and as a preliminary communication in J. Am. Chem. Soc. **85**, 643 (1963). See also S. Wolfe, U.S. Patent No. 3,311,638 (1967).

[2]Department of Chemistry, Queen's University, Kingston, Ontario. Correspondence concerning this paper should be addressed to this author.

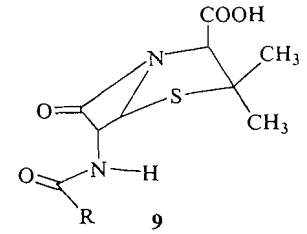
[3]Bristol Laboratories of Canada, Ltd., Candiac, Quebec.

[4]The total synthesis of the cephalosporins has been reported by R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbrüggen, J. Am. Chem. Soc. **88**, 852 (1966).

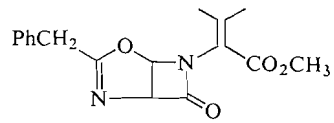
[5]A scheme analogous to this has been achieved in a model system (4).

The synthetic problem associated with the introduction of a substituent onto an unactivated methyl group has received much attention in recent years, especially in connection with substitution at C-18 and C-19 of the steroid nucleus. Success has usually been achieved by the use of reactions in which a substituent is transferred to methyl from a reactive center generated within the same molecule (5, 6).

When penicillin is viewed in three dimensions, it can be seen (**9** (7)) that the carboxyl group, the sulfur atom, and the amide hydrogen of the side chain are potential sites from which attack on a methyl group might be initiated. Conversion of the carboxyl group into radical (anodic oxidation) or cationic (lead tetraacetate) forms does not, however, lead to intramolecular reactions. Instead, decarboxylation is the major process observed (8). Treatment of the *N*-chloro derivative of penicillin G methyl ester with triethylamine led to loss of the sulfur and formation of the oxazoline **10** (9). Morin and co-workers (10) have

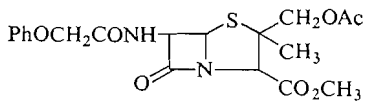


9

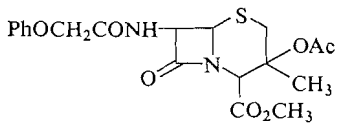


10

been successful in using the sulfur atom to initiate attack on a methyl group. Heating the methyl ester of penicillin V sulfoxide to reflux in acetic anhydride gave **11** and **12** in 40% and 20% yields, respectively (cf. ref. 11).



11



12

In this article we report a different kind of rearrangement of the thiazolidine ring from that proposed in Chart 1. Though the reaction does not involve the methyl groups directly, as a consequence of the rearrangement it appears possible to introduce substituents onto these groups and thus arrive at **8** in an indirect manner. The rearrangement was discovered in the course of experiments with penicillin acid chlorides.

Before it was shown (12, 13) that the cephalosporin nucleus contains a 6-membered ring, Abraham and Newton had suggested (14) that these compounds contained a penicillin nucleus with an additional acetoxy group α to the carboxyl function. Though this seemed incompatible with the ultraviolet (u.v.) spectrum of cephalosporins (λ_{max} ~260 mμ), we thought that access to such a compound might be had by Baeyer–Villiger oxidation of a ketene dimer potentially available from a penicillin acid chloride by treatment with a tertiary amine (e.g. **13** → **15**).

The preparation of analytically pure acid chlorides of penicillins was claimed by Villax in a British patent in 1956 (15). Because of the instability of penicillins in acid media (see above; also ref. 16), penicillin acid chlorides would not be expected to be particularly stable and our own work did not afford analytically pure compounds. However, by careful control of the stoichiometry (Chart 2) it is possible, using thionyl chloride, to observe conversion in solution of a penicillin to a penicillin acid chloride.

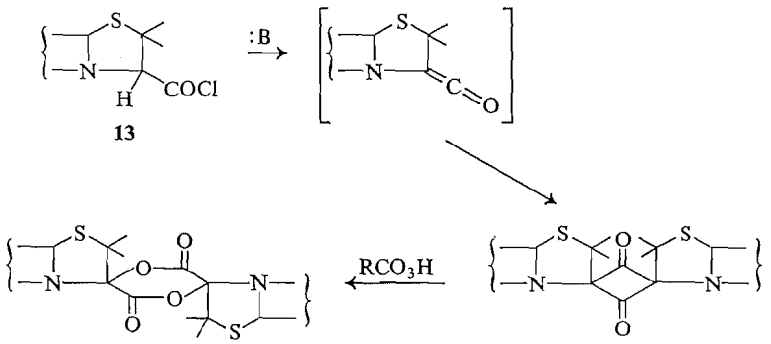
A $RCOOH + SOCl_2 \rightarrow RCOCl + SO_2 + HCl$

B $RCOOM + SOCl_2 \rightarrow RCOCl + SO_2 + MCl$

C $RCOOH \cdot H_2O + SOCl_2 \rightarrow RCOCl + 2\,SO_2 + 3\,HCl$

Chart 2. Stoichiometry of the conversion of acids to acid chlorides. A, Carboxylic acid to acid chloride; B, alkali metal salt to acid chloride; C, carboxylic acid hydrate to acid chloride.

Stoichiometric reaction of thionyl chloride with a carboxylic acid (A of Chart 2) leads to an acid chloride together with SO_2 and HCl. These would cause rapid destruction of a penicillin if permitted to remain in the reaction mixture. Therefore, at least 2 mole-equivalents of pyridine are added per mole of carboxylic acid. Most penicillins are available in the form of the sodium or potassium salts. As shown in B, at least 1 mole-equivalent of pyridine is then necessary. Occasionally a penicillin acid is obtained as a



13 15 14

crystalline hydrate. As shown in C, this requires the use of 2 mole-equivalents of thionyl chloride and at least 5 mole-equivalents of pyridine. Using the quantities indicated by the appropriate equation of Chart 2, penicillins can be smoothly converted to their acid chlorides at $-20°$ in CH_2Cl_2, and the progress of the reactions followed by infrared spectroscopy.

The carbonyl region of the potassium salt of a penicillin shows absorption at 5.6 μ (β-lactam), 6.0 μ (amide I), and 6.2 μ (CO_2^-). The addition of thionyl chloride (1 mole-equivalent) to a suspension of a potassium salt (1 mole-equivalent) in methylene chloride containing pyridine (1 mole-equivalent) leads to immediate dissolution of the penicillin and the bright-yellow solution then shows absorption at 5.6, 5.8, and 6.0 μ. After 3 h at -20 °C the infrared (i.r.) spectrum shows absorption at 5.5 μ (COCl), 5.6 μ, and 6.0 μ. These spectral changes are consistent with the reaction sequence,

$$\text{PenCOOK} \xrightarrow[\text{fast}]{SOCl_2/\text{pyr}} \text{Pen-COOSOCl} \xrightarrow[\text{slow}]{\text{pyr}} \text{Pen-COCl} + \text{pyr}\cdot SO_2$$

Anhydride formation (17) was not observed so long as 1 mole-equivalent of thionyl chloride was used per mole of penicillin.

For the isolation of a penicillin acid chloride, salts were precipitated by addition of diethyl ether; after filtration and evaporation of the solvent *in vacuo* below 30°, the residue was triturated with benzene, filtered again, and the benzene solution was lyophilized. A sample of α-phenoxyethylpenicillin acid chloride prepared in this way gave a chlorine analysis of 7.5%; the theoretical value was 9.3%.

Addition of excess pyridine had no effect on penicillin acid chloride reaction mixtures. Triethylamine is a stronger base than pyridine and when this was added gradually at $-20°$ to a penicillin acid chloride reaction mixture[6] dehydrohalogenation of the acid chloride did not occur until more than *n* mole-equivalents of triethylamine had been added, where *n* was the number of mole-equivalents of pyridine used in the preparation of the acid chloride.[7]

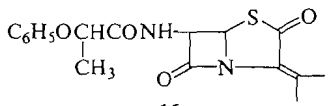
When 2 mole-equivalents of triethylamine were gradually added to an α-phenoxyethylpenicillin acid chloride reaction mixture prepared by method B, a new spectrum appeared which now showed strong absorption at 5.5, 5.9, and 6.0 μ and medium absorption at 6.1 μ. The appearance of the spectrum indicated that treatment of the acid chloride with triethylamine under these conditions had not caused loss of the β-lactam. Evaporation of the reaction mixture and trituration of the residue with ethanol then gave a crystalline compound, m.p. 150–152°, whose i.r. spectrum (in methylene chloride) showed the same group of peaks in the carbonyl region as the reaction mixture and no peaks characteristic of either a ketene or the expected ketene dimer. The compound had an apparent molecular weight in a Signer determination of 369 and analysis indicated the molecular formula $C_{17}H_{18}N_2O_4S$. Since α-phenoxyethylpenicillin is $C_{17}H_{20}N_2O_5S$, the new compound was derived from the penicillin by loss of water and it was designated *anhydro-α-phenoxyethylpenicillin.*

Anhydro-α-phenoxyethylpenicillin was neutral and had no antibacterial activity. The compound

[6]Isolation of the acid chlorides and then treatment with triethylamine led to the formation of tars.

[7]This is a consequence, of course, of the fact that triethylamine is a stronger base than pyridine.

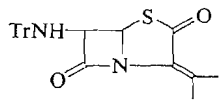
had ultraviolet absorption at 269 mμ (ε_{max} ($CHCl_3$) = 12 000) and this, together with the i.r. absorption at 5.9 and 6.1 μ, suggested the presence of an α,β-unsaturated carbonyl system. The i.r. absorptions at 5.5 and 6.0 μ were assigned to the β-lactam and the amide. The spectrum also showed N—H absorption at 3.0 and 6.6 μ. The nuclear magnetic resonance (n.m.r.) spectrum showed all 18 protons clearly, and comparison with the n.m.r. spectrum of α-phenoxyethylpenicillin revealed that the proton α to the carboxyl group had been lost in the formation of the anhydropenicillin; in addition, the absorption of the methyl groups of the nucleus had shifted from τ 8.50 and 8.58 to τ 7.83 and 7.92. All of these data are consistent with the structure **16** for anhydro-α-phenoxyethylpenicillin and, in agreement with this structure, acetone was obtained in 44% yield upon ozonolysis of the compound. No acetone was obtained upon ozonolysis of the parent penicillin.



16

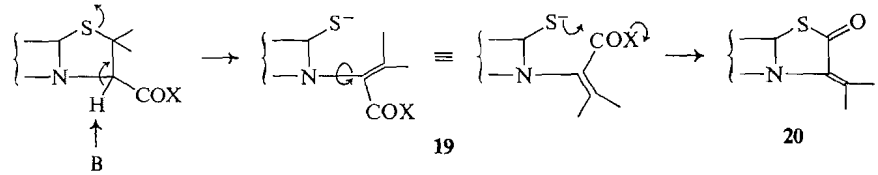
Because anhydropenicillins are useful intermediates for further work, a study was made of the scope of the rearrangement. It was shown that, provided the conditions described above were rigorously followed, the rearrangement is a general one for penicillins. However, the isolation of an anhydropenicillin had to be performed with care. Most anhydropenicillins were only slightly soluble in ethyl ether, ethanol, and isopropanol and these were excellent solvents for either precipitation or recrystallization of anhydropenicillins after removal of pyridine, triethylamine, and the various salts. The methylene chloride reaction mixtures were shaken with dilute HCl, water, 10% sodium bicarbonate solution, and then with water again. Evaporation of the dried methylene chloride solutions and treatment of the residue with one of the above solvents gave crystalline anhydropenicillins.

The isolated yields of anhydropenicillins were not high (ca. 20–30%). In some cases this could be attributed to the occurrence of further reactions during the isolation procedure. Thus, as noted above, the i.r. spectrum of pure anhydro-α-phenoxyethylpenicillin in methylene chloride was identical in the carbonyl region with the spectrum of the reaction mixture. Nevertheless, a second crystalline compound was also obtained when the products were isolated.[8] In addition, the isolation of **17**, the anhydro derivative of 6-*N*-tritylaminopenicillanic acid had to be performed without water treatment. Shaking the methylene chloride reaction mixture with water caused disappearance of the anhydropenicillin and formation of a new β-lactam-containing compound (18).



17

It is suggested that the formation of an anhydropenicillin proceeds by opening of the thiazolidine ring (**18** → **19**; X = Cl)[9] followed by recyclization at the acyl carbon (**19** → **20**).



18 **19** **20**

The ring-opening depicted in **18** → **19** is an example of a reverse Michael reaction. It was thought that this step would be facilitated by the use of other carboxy derivatives in which the hydrogen α to the carboxyl group is activated towards abstraction as a proton. To test this, a number of penicillin esters and mixed anhydrides were subjected to base treatment with the results summarized in Table I. These results show that carbonic and sulfonic anhydrides are not as

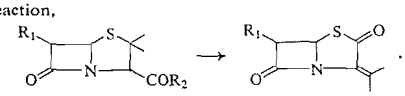
[8]This compound is dimeric (see Experimental).
[9]See, for example, N. J. Leonard and R. Y. Ning, J. Org. Chem. **32**, 677 (1967).

TABLE I

The base-catalyzed rearrangement of carboxy activated penicillins to anhydropenicillins*

R_1	R_2	Conditions	% yield of anhydropenicillin
$C_6H_5OCHCONH$ (CH$_3$)	Cl	a†	22
$C_6H_5OCHCONH$ (CH$_3$)	OTs	a	12
$C_6H_5OCHCONH$ (CH$_3$)	SO_2CH_3	a	6
$C_6H_5OCHCONH$ (CH$_3$)	$OCOC_2H_5$	a	5
CH_3OCMe_2CONH	$OCOC_2H_5$	b‡	Not determined
$C_6H_4(CO)_2N$	Cl	a	28
$C_6H_4(CO)_2N$	OCH_3	c§	0
$C_6H_4(CO)_2N$	OCH_3	d‖	0

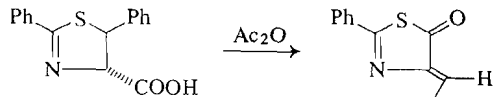
*The reaction,



†Triethylamine in methylene chloride.
‡Sodium methoxide in methylene chloride.
§Sodium hydride in tetrahydrofuran.
‖Potassium *t*-butoxide in tetrahydrofuran – *t*-butanol.

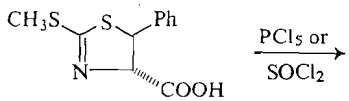
effective substrates for the rearrangement as the acid chlorides when the base is a tertiary amine, and that methyl esters are less reactive, even towards strong base.[10]

[10]Transformations analogous to the anhydropenicillin rearrangement have been noted previously. Lur'e and Gatsenko (19) converted the acid **21** into the thiazolinone **22** by treatment with acetic anhydride, an experiment which was confirmed by Sicher and co-workers (20).

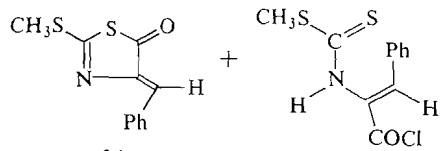


21 **22**

The action of $SOCl_2$ or PCl_5 on the acid **23** produces the thiazolinone **24** and (apparently) the acid chloride **25** (21).



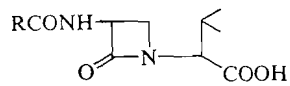
23



24 **25**

We turn now to a consideration of some physical and chemical properties of anhydropenicillins. The most noteworthy feature of the compounds is their chemical stability. Though the i.r. spectra seem to indicate that both carbonyl groups of the bicyclic system are under considerable strain, the anhydropenicillins are extraordinarily stable. They are recovered unchanged after prolonged refluxing in alcohol, aqueous dioxane, toluene, xylene, or after being melted.

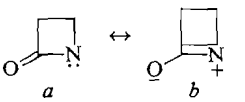
Several groups of workers have sought relationships between the carbonyl stretching band of the β-lactam and the antibacterial activity of a penicillin (7, 22, 23). Most penicillins show i.r. absorption near 5.62 μ; monocyclic β-lactams and desthiopenicillins (**26**) absorb in the region



26

5.69–5.78 μ, while fused thiazolidine β-lactams absorb at 5.65 μ (24). The penicillins are considerably less stable to hydrolysis than any of the simpler β-lactam-containing compounds (22). Excluding the cephalosporins from this discussion, of those compounds containing the β-lactam

ring, the penicillins are characterized by the highest antibacterial activity, the lowest wavelength carbonyl stretching band, and the highest rate of hydrolysis. It was suggested some years ago (25) that the latter two properties are a reflection of the extent of delocalization of charge in the cyclic amide (e.g. **27**); increased participation by **27***b* would be associated with an i.r.



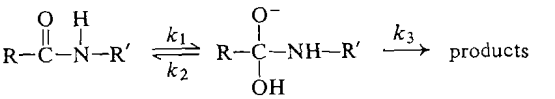
a *b*

27

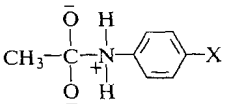
shift to higher wavelengths, greater stability to hydrolysis (25), and with decreased basicity of the nitrogen atom (26).

Were the anhydropenicillins to display properties consistent with those just discussed, the fact that their i.r. absorption occurs at 5.5 μ would lead to the predictions that the anhydropenicillins should be labile and biologically active. Neither of these predictions is correct.[11] Consequently, though the position of the β-lactam band in the infrared indicates a high degree of true carbonyl character (i.e. **27***a*), there must be an additional factor which counterbalances this during the hydrolysis.

It is known that the hydrolysis of amides (29) and lactams (30) involves the formation of tetrahedral intermediates which then partition themselves between reactants and products. In a study of the concurrent alkaline hydrolysis and isotopic oxygen exchange of a series of *p*-substituted acetanilides Bender and Thomas (31) found that electron-releasing substituents cause an increase in k_3/k_2, the ratio of the rate constants for hydrolysis and exchange,

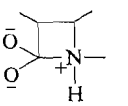


i.e., that stabilization of an anionic leaving group is not a principal factor in the breakdown of the tetrahedral intermediate. They interpreted their results to mean that a dipolar ion, e.g. **28**, is formed whose decomposition is rate limiting and whose concentration is increased as the basicity of the nitrogen atom of the anilide increases.



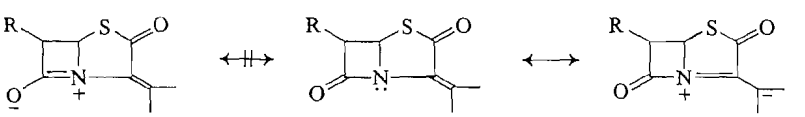
28

The same explanation, rather than a more qualitative "relief of steric strain" argument accounts nicely for the relationship already noted between the ease of hydrolysis of a β-lactam and the C=O stretching band in the i.r. spectrum. Suppression of resonance in the β-lactam ought to be associated with an increase in the basicity of the nitrogen atom and concomitant increase during hydrolysis of the concentration of a dipolar ion such as **29**. In terms of this explanation it is



29

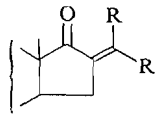
evident that the nitrogen atom of an anhydropenicillin is less basic than anticipated. The most reasonable explanation of this and of the i.r. absorption at 5.5 μ is that the electron pair on the nitrogen atom is delocalized *in the ground state*[12] into the adjacent α,β-unsaturated system; i.e.,



[11]Obviously it would be naive to consider that the stability of the β-lactam is the only factor affecting the antibacterial activity of a penicillin derivative. Structure-activity studies (1, 7, 27), as well as studies of the mode of action of penicillin (28) indicate that the carboxyl group is essential for activity. The absence of a carboxyl group in the anhydropenicillins is, therefore, consistent with their lack of activity.

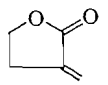
[12]A similar proposal was made by Abraham and Newton (12) for the *excited state* of Cephalosporin C in order to account for the u.v. absorption at 260 mμ. More recent work (32, 33) indicates that this is only partly correct, the sulfur atom also participating in the chromophore of Cephalosporin C.

That the electron pair on the nitrogen atom is also delocalized *in the excited state* can be seen from the following argument. The 16-alkylidene-dehydroisoandrosterones **30***a* and **30***b* show u.v.

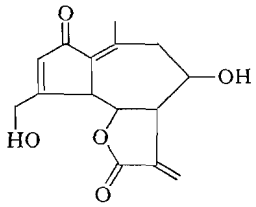


30 (*a*, R = H; *b*, R = CH_3)

absorption at 228 mμ and 250 mμ, respectively (34, 35). The replacement of the carbon α to carbonyl by oxygen leads to a decrease in both the wavelength and the intensity of the absorption. The unsaturated γ-lactone **31** showed only end absorption (ε 2400 at 230 mμ) (36). The unsaturated γ-lactone system of lactucin (**32**) was found to absorb at 208 mμ (ε 8600) (37). Replace-

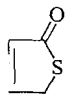


31

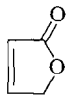


32

ment of the ring oxygen of an unsaturated γ-lactone by sulfur causes a small bathochromic shift. The thiolactone **33** absorbs at 220 mμ (38, 39) and the butenolide **34** has λ_{max} 214 mμ (40).



33



34

The introduction of substituents onto **33** results in a bathochromic shift of the low wavelength maximum (41) but there is also a substituent independent maximum at 265 mμ. The nature of this latter absorption has not been explained, but by analogy with the work already quoted (32, 33) this may be associated with an interaction between sulfur and the double bond.

If the β-lactam nitrogen does not participate in the chromophore, the u.v. maximum of an anhydropenicillin should be

$$208(\lambda_{max}\ \mathbf{32}) + 22(\lambda_{max}\ \mathbf{30}b - \lambda_{max}\ \mathbf{30}a) + 6(\lambda_{max}\ \mathbf{33} - \lambda_{max}\ \mathbf{34}) = 236\ m\mu$$

The bathochromic effect of nitrogen is thus ~34 mμ. This is a reasonable value (ref. 40, pp. 109, 243). Whether there is a second maximum near 270 mμ associated with the sulfur atom (41) cannot be stated at the present time.

Experimental

Melting points were taken on a Fisher–Johns block and are uncorrected. Infrared (i.r.) spectra were obtained on a Baird spectrometer, u.v. spectra on a Beckmann DK-2 instrument, and nuclear magnetic resonance (n.m.r.) spectra on a Varian A60 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Microanalyses are by R. M. Downing and his staff at Bristol Laboratories, Syracuse, New York. All solvents and chemicals were reagent grade except for the penicillins. Penicillin G and α-phenoxyethylpenicillin[13] were the commercially available materials; other penicillins were synthesized from 6-aminopenicillanic acid as described below. Thionyl chloride was purified by simple distillation; material purified by distillation from quinoline and then from linseed oil (42) did not seem more effective.

α-Phenoxyethylpenicillin Acid Chloride

(*a*) Potassium α-phenoxyethylpenicillin (12.1 g, 0.03 mole) was suspended in methylene chloride (100 ml), pyridine (2.4 ml, 0.03 mole) was added, and the mixture was cooled to −20° (internal temperature). A solution of thionyl chloride (3.6 g, 0.03 mole) in methylene chloride (50 ml) was then added in a steady stream. The internal temperature rose to −10° and the penicillin dissolved to produce a clear colorless solution. With continued external cooling the internal temperature was lowered to −20° and after 5 min the reaction mixture had begun to turn yellow. The temperature was maintained at −20° and stirring was continued for 3½ h by which time a deep yellowish-orange suspension was present. When an aliquot was removed for i.r. analysis and allowed to warm to room temperature a clear orange solution was obtained. This solution showed λ_{max} 5.5, 5.6 (sh), and 6.0 μ. The reaction mixture was then diluted with 300 ml of dry ether to precipitate salts, the cooling bath was removed, and when the internal temperature had reached 10°, the mixture was filtered and the filtrate was evaporated to dryness to produce 6.1 g (53%) of a yellow fluff. The i.r. spectrum of this fluff showed weak absorption at 5.8 μ, indicating that some hydrolysis had occurred during the work-up.

Anal. Calcd. for $C_{17}H_{19}N_2O_4ClS$: C, 53.4; H, 4.97. Found: C, 54.6; H, 5.46.

(*b*) The experiment was repeated as described in (*a*) and the substance remaining after evaporation of the ether was dissolved in benzene, the solution was filtered to remove some insoluble material, and the filtrate was then frozen and stored in a deep freeze for three days. The benzene was then removed by lyophilization to give a pale-yellow solid. The i.r. spectrum of this solid showed λ_{max} ($CHCl_3$) 5.5, 5.6 (sh), 5.8 (w), 6.0 μ.

Anal. Calcd. for $C_{17}H_{19}N_2O_4ClS$: Cl, 9.3. Found: Cl, 7.5.

[13]The trademark of Bristol Laboratories for α-phenoxyethylpenicillin is Syncillin.

The purpose of this experiment was to demonstrate that, after its preparation and isolation, the acid chloride could be stored in a convenient form until needed.

Anhydro-α-phenoxyethylpenicillin

(a) Via the Acid Chloride

Run 1—Potassium α-phenoxyethylpenicillin (365 g, 0.91 mole) was suspended in methylene chloride (3 l) containing pyridine (79 g, 1 mole). The mixture was stirred, cooled to −20°, and thionyl chloride (119 g, 1 mole) in methylene chloride (600 ml) was then added as rapidly as possible. The reaction mixture was stirred for 3 h at −20° and was then treated during 1 h with a solution of triethylamine (184 g, 1.82 moles) in methylene chloride (500 ml). Ten minutes after the addition was complete the i.r. spectrum of an aliquot showed λ_{max} 5.5, 5.9, 6.0, and 6.1 μ. The reaction mixture was then warmed to room temperature and the solvent was removed under reduced pressure. The black gum which remained solidified upon trituration with ethanol (2500 ml). Filtration, concentration of the filtrate to 1500 ml and then storage at 0° produced a crystalline compound which was collected and recrystallized thrice from acetone–water to give 20.0 g, m.p. 150–152°. The insoluble material from the ethanol trituration was extracted with acetone (2000 ml); this extract was concentrated to 500 ml and then diluted with 3000 ml of water. The precipitated solid was collected and washed with water. After drying *in vacuo* over phosphorous pentoxide, the solid weighed 56.0 g and melted at 142–145°. Three recrystallizations from acetone–water gave a tan solid; this was suspended in cold ethanol and the suspension filtered to produce 45.5 g of a white solid, m.p. 150–150.5°. The total yield of pure material was thus 65.6 g (21%).

Anal. Calcd. for $C_{17}H_{18}N_2O_4S$ (mol. wt., 346): C, 59.0; H, 5.21; N, 8.10. Found (mol. wt., 369, Signer): C, 59.16; H, 5.25; N, 8.31.

Run 2—Potassium α-phenoxyethylpenicillin (100 g, 0.25 mole) and triethylamine hydrochloride (35.0 g, 0.25 mole) were suspended in methylene chloride (1000 ml) and the mixture was stirred at 25° for 1 h to effect the conversion of the potassium salt to the triethylamine salt. The mixture was then cooled to −30° and pyridine (20 ml, 0.25 mole) was added followed with rapid stirring, by thionyl chloride (24.5 ml, 0.30 mole). The temperature was maintained at −25° to −30° for 1 h and the bright-orange suspension was then treated with 70.0 ml (0.50 mole) of triethylamine. After 10 min the mixture was warmed rapidly to room temperature and glacial acetic acid (30 ml, 0.5 mole) was added. The solution was washed with water, dried over anhydrous sodium sulfate, diluted with ether (4000 ml) and, after treatment with activated carbon, evaporated to dryness. The gum thus obtained was dissolved in hot absolute ethanol (100 ml). Cooling of this solution produced 20.4 g of yellow crystals. Recrystallization from boiling ethanol containing activated carbon gave 18.8 g (22%) of anhydro-α-phenoxyethylpenicillin, m.p. 148–150°.

(b) Via the Methanesulfonic Anhydride

Potassium α-phenoxyethylpenicillin (40.2 g, 0.10 mole) was suspended in methylene chloride (600 ml) and this suspension was treated during a 30 min period with a solution of methanesulfonyl chloride (11.5 g, 0.10 mole) in methylene chloride (50 ml). The reaction mixture was refluxed overnight and then cooled to 0° and treated dropwise over a 45 min period with a solution of triethylamine (10.1 g, 0.10 mole) in methylene chloride (50 ml). After continued stirring at 0° for 1 h and then at 30° for 2 h, water (200 ml) was added and the methylene chloride layer was separated, dried over anhydrous sodium sulfate, and then diluted with dry ether (3000 ml). After treatment with activated carbon the solution was concentrated and the residual oil was dissolved in absolute ethanol. After 48 h at 0°, the crystalline anhydro-α-phenoxyethylpenicillin, m.p. 148–150°, was collected. It weighed 2.0 g (6%).

(c) Via the Ethoxycarbonic Anhydride

Potassium α-phenoxyethylpenicillin (40.3 g, 0.10 mole) was suspended in methylene chloride (400 ml) and 13.8 g (0.10 mole) of triethylamine hydrochloride were added. The mixture was stirred for 1 h at 26°, then cooled to −3°, and ethyl chloroformate (9.52 ml, 0.10 mole) was added dropwise over a 5 min period. Stirring was continued, and after 15 min, the addition of triethylamine (14.1 ml, 0.10 mole) in methylene chloride (50 ml) was commenced. The addition required 1 h, the temperature being maintained at −3°. After an additional 2 h glacial acetic acid (11.4 ml, 0.20 mole) in methylene chloride (50 ml) was added dropwise. The temperature of the reaction mixture was then raised to 27° and maintained there for 35 min. Water (200 ml) was then added and the organic phase was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure at 41° to an orange-brown gum. Crystallization from absolute ethanol afforded 1.77 g of anhydro-α-phenoxyethylpenicillin (5%), m.p. 146–148°. Found: C, 58.65; H, 5.47.

(d) Via the p-Toluenesulfonic Anhydride

Potassium α-phenoxyethylpenicillin (40.3 g, 0.10 mole) and *p*-toluenesulfonyl chloride (19.19 g, 0.10 mole) were stirred in methylene chloride (550 ml) for 16 h at 30–31°. Precipitation of potassium chloride during this period indicated that the mixed anhydride had formed. A solution of triethylamine (14.1 ml, 0.10 mole) in methylene chloride (50 ml) was then added during 20 min. The temperature rose to 34° and the colorless solution turned greenish-black. Stirring was continued for 7 h at 30° and the mixture was then washed with water (200 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness at 40°. The residue was triturated with ethanol (100 ml) and the mixture was then kept at 5° for 24 h. There was then obtained by filtration 4.10 g (12%) of anhydro-α-phenoxyethylpenicillin, m.p. 140–144°.

Anhydropenicillin G

Potassium penicillin G (potassium benzylpenicillin) (37.2 g, 0.1 mole) was suspended in methylene chloride (250 ml) containing pyridine (8.9 ml, 0.1 mole). The mixture was cooled to −20° and thionyl chloride (8 ml, 13.3 g, 0.11 mole) was added over a 2 min period. Stirring was continued for 3 h at −20°. The temperature was then lowered to −30° and triethylamine (34 ml, 24.5 g, 0.25 mole) was added during 15 min. The temperature was allowed to rise to −20° during the next 30 min and was kept at −20° for 1.5 h and then allowed to rise to 0° during the next hour. Water (250 ml) was then added and the organic phase was separated, dried over an-

hydrous sodium sulfate, and evaporated to dryness. The black sticky mass thus obtained was dissolved in ethanol (200 ml) and this solution was kept overnight at 0°. The crystalline material which was deposited during this period was collected and recrystallized from aqueous acetone to yield 2.42 g (8%) of anhydropenicillin G, m.p. 156–158° (decomp.); λ_{max} (KBr): 5.5, 5.9, 6.0, 6.1 μ.

Anal. Calcd. for $C_{16}H_{16}N_2O_3S$: C, 60.74; N, 5.02; N, 8.86. Found: C, 60.82; H, 5.17; N, 8.85.

Anhydro-6-N-phthaloylaminopenicillin

The following procedure for the preparation of 6-*N*-phthaloylaminopenicillanic acid is adapted from one described by Nefkens and co-workers (43).

6-Aminopenicillanic acid (38.6 g, 0.18 mole) was dissolved in an aqueous solution (270 ml) containing sodium carbonate (19.1 g, 0.18 mole), and finely powdered *N*-carbethoxyphthalimide (43) (44.4 g, 0.20 mole) was added in one portion. The mixture was stirred vigorously at room temperature for 3.5 h and was then extracted twice with 100 ml portions of methylene chloride. The combined methylene chloride extracts were dried over anhydrous sodium sulfate and the solvent removed to give 17.7 g of unreacted *N*-carbethoxyphthalimide. The aqueous phase was diluted with acetone (80 ml) and was acidified to pH 3 with 2 *N* hydrochloric acid. The product crystallized immediately and was collected and washed with water. Concentration of the mother liquor produced a second crop of material which was also collected. The two crops (26.4 g) were combined and recrystallized from aqueous acetone to give 22.3 g of 6-*N*-phthaloylaminopenicillanic acid, m.p. 178–180° (decomp.). Based on the unreacted *N*-carbethoxyphthalimide the yield was 54%.

Anal. Calcd. for $C_{16}H_{14}N_2O_5S$: C, 55.49; H, 4.05. Found: C, 55.37; H, 4.15.

(a) Via the Acid Chloride

6-*N*-Phthaloylaminopenicillanic acid (20.0 g, 58 mmoles) and pyridine (10.17 ml, 0.12 mole) were dissolved in methylene chloride (230 ml). The solution was cooled to −20° and a solution of thionyl chloride (4.51 ml, 7.49 g, 63 mmoles) in methylene chloride (35 ml) was added as rapidly as possible. The reaction mixture was stirred at −15° to −20° for 3 h and was then treated during 0.5 h with a solution of triethylamine (24.3 ml, 17.5 g, 0.17 mole) in methylene chloride (70 ml). The temperature was brought to 20° and the reaction mixture was washed successively with 5% hydrochloric acid, 5% sodium bicarbonate and water, and then dried over anhydrous magnesium sulfate. Evaporation of the methylene chloride gave 16.05 g of a neutral residue. Two recrystallizations from 90% acetone gave 5.35 g (28%) of anhydro-6-*N*-phthaloylaminopenicillin, m.p. 236–237°.

Anal. Calcd. for $C_{16}H_{12}N_2O_4S$: C, 58.53; H, 3.66. Found: C, 58.84; H, 3.87.

Anhydro-α-methoxyisobutyrylpenicillin

A solution of α-methoxyisobutyric acid (11.8 g, 0.1 mole) in a mixture of acetone (20 ml) and dioxane (80 ml) was treated successively at −5° with triethylamine (15 ml, 10.8 g, 0.11 mole) and then isobutyl chloroformate (13.6 g, 0.1 mole) in dioxane (15 ml). To the mixed anhydride were added rapidly a solution of 6-aminopenicillanic acid (21.6 g, 0.1 mole) in water (100 ml) and triethylamine (15 ml, 0.11 mole), the temperature being maintained below 10°. The clear solution was stirred at 10° for 0.5 h, at 20° for 2 h, and was then diluted with 250 ml of water and extracted with ether, the ether extract being discarded. The aqueous layer was covered with fresh ether (300 ml), cooled, and acidified to pH 2 with sulfuric acid. The ether layer was separated, washed with cold water, dried over anhydrous sodium sulfate, and treated with 50 ml of a 40% solution of potassium 2-ethylhexanoate in *n*-butanol. The potassium 6-α-methoxyisobutyrylpenicillin was collected, triturated with ether and then dried *in vacuo* over phosphorous pentoxide. The yield was 24.5 g (70%), m.p. 234–236° (decomp.).

Anal. Calcd. for $C_{13}H_{20}N_2O_5SK$: C, 43.92; H, 5.67. Found: C, 43.85; H, 5.76.

The penicillin (6.33 g, 0.018 mole) was dissolved in methylene chloride (80 ml) containing triethylamine (2.67 ml, 0.019 mole) and the solution was cooled to 0°. Then ethyl chloroformate (1.90 ml, 0.02 mole) in methylene chloride (20 ml) was added dropwise during 5 min to form the mixed anhydride. Stirring was continued for 15 min at 0° and then powdered freshly prepared sodium methoxide (2.2 g, 0.04 mole) was added all at once. Stirring was continued for 2½ h at which time ice-cold pH 6 phosphate buffer (100 ml) was added. The layers were separated and the aqueous layer extracted with 50 ml of methylene chloride. The combined organic extract, after drying over anhydrous sodium sulfate, was evaporated to give a yellow gum. This gum could not be crystallized but the presence of anhydropenicillin was evident from the u.v. absorption at 269 mμ and i.r. absorption at 5.5, 5.9, and 6.1 μ.

6-(2-Hydroxy-1-naphthalamino)penicillanic Acid

To a suspension of 6-aminopenicillanic acid (4.32 g, 0.02 mole) in methanol (100 ml) was added 2-hydroxy-1-naphthaldehyde (44) (3.44 g, 0.02 mole). The mixture was stirred overnight, by which time dissolution of the 6-aminopenicillanic acid was complete, and the solvent was then removed under reduced pressure. Trituration with ether afforded the Schiff base as yellow crystals, m.p. 174–176° (decomp.); the yield was 6.6 g (90%).

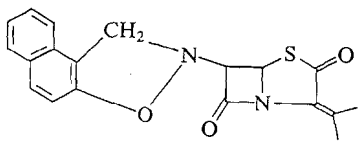
Anal. Calcd. for $C_{19}H_{18}N_2O_4S$: C, 61.60; H, 4.89. Found: C, 61.40; H, 4.95.

Anhydro Derivative of 6-(2-Hydroxy-1-naphthalamino)-penicillanic Acid

The Schiff base (18.5 g, 0.05 mole) was suspended in methylene chloride (200 ml) containing pyridine (8.8 ml, 0.106 mole) and triethylamine (7 ml, 0.05 mole). The resulting solution was cooled to −20° and thionyl chloride (3.9 ml, 0.054 mole) was added as rapidly as possible. The reaction mixture was stirred for 3 h at −15 to −20° and was then treated during ½ h with triethylamine (14 ml, 0.1 mole) in methylene chloride (30 ml). Evaporation then afforded a crystalline solid which was isolated by trituration with ethanol and was recrystallized from aqueous acetone. The yield was 4.2 g (24%); m.p. 219–221° (decomp.).

Anal. Calcd. for $C_{19}H_{16}N_2O_3S$: C, 64.77; H, 4.54. Found: C, 64.47; H, 4.71.

Because the compound is a white solid and shows no i.r. absorption near 3 μ it is considered to have the cyclic structure **35**.



35

Isolation of Anhydro-α-phenoxyethylpenicillin "Dimer"

Run 1

Potassium α-phenoxyethylpenicillin (12.1 g, 0.03 mole) was converted to the acid chloride in the usual manner (*vide supra*) in methylene chloride (150 ml) and excess triethylamine was then added. When the i.r. spectrum of the reaction mixture showed conversion to the anhydropenicillin to be complete the mixture was warmed to room temperature, diluted with ether (800 ml), and filtered. The filtrate was evaporated to dryness, the residue triturated with tetrahydrofuran (50 ml), filtered, and re-evaporated to give 8.2 g of a brown solid (A) which smelled of pyridine.

One gram of A was dissolved in isopropyl alcohol (40 ml) and the solution was treated with decolorizing charcoal and then concentrated to 20 ml. Cooling to −10° produced a solid (B) which was collected. The filtrate from B upon dilution with water (75 ml) gave 0.2 g of a substance C. Heating B to reflux with isopropyl alcohol (40 ml) afforded an additional 0.29 g of (insoluble) C; identity of the two fractions was established by i.r. (*vide infra*).

One-half gram of A was dissolved in absolute ethanol (5 ml) and the solution was cooled to −10° to give anhydro-α-phenoxyethylpenicillin. This was collected and the filtrate was diluted with water (25 ml) to precipitate solid C (identified by its i.r. spectrum).

Run 2

Eighty grams of crude anhydro-α-phenoxyethylpenicillin prepared by the method of run 2 (*vide supra*) were recrystallized from boiling ethanol (2500 ml) and, after the purified anhydropenicillin was collected, the mother liquor was concentrated to 1000 ml and stored at 0° for two weeks. Six grams of substance C were then obtained by filtration.

A saturated solution of C (0.1 g) in methylene chloride (25 ml) was poured through a column of alumina (Woelm, activity III). Evaporation of the eluate and recrystallization from methylene chloride gave pale-yellow needles, m.p. 262–264°. The i.r. spectrum of this compound shows λ_{max} (KBr) 3.12, 3.27, 5.53, 5.93, 5.98, 6.03, 6.06, 6.10, 6.27, 6.60, 6.68 μ.

Anal. Calcd. for $C_{32}H_{32}N_4O_7S$ (mol. wt., 616.68): C, 62.32; H, 5.23; N, 9.09; S, 5.19. Found (*m/e*,[14] 616): C, 62.55, 62.20; H, 5.66, 5.48; N, 8.56, 8.84, 8.75; S, 4.98, 5.16.

Ozonolysis of Anhydro-α-phenoxyethylpenicillin

A solution of anhydro-α-phenoxyethylpenicillin (99 mg, 0.29 mmoles) in methylene chloride (10 ml) was cooled to 0° and treated for 20 min at a rate of 0.02 ft³/min with a stream of dry air containing ozone, generated at 90 V in a Welsbach ozonator. The reaction mixture, which contained a heavy precipitate, was rinsed into a distillation flask with water (12 ml), and zinc dust (100 mg) was added. All but 1 ml of the methylene chloride was removed by careful distillation and this distillate was discarded. Then the remainder of the methylene chloride and ca. 2 ml of water were distilled out and this second distillate was treated with 2.4 ml of a 2,4-dinitrophenylhydrazine reagent prepared in the usual manner (45). The mixture was warmed to distil out the remaining methylene chloride and then cooled in ice. A crystalline precipitate, 35 mg, m.p. 116–120°, was then obtained. Recrystallization from aqueous ethanol gave 30 mg (44%) of acetone 2,4-dinitrophenylhydrazone, m.p. 123.0–124.0°, identified by comparison with an authentic specimen.

[14]We are grateful to Professor D. B. MacLean for this determination.

1. H. T. Clark, J. R. Johnson, and R. Robinson (*Editors*). The chemistry of penicillin. Princeton University Press, Princeton, New Jersey. 1949.
2. G. G. F. Newton and E. P. Abraham. Biochem. J. **62**, 651 (1956).
3. B. Loder, G. G. F. Newton, and E. P. Abraham. Biochem. J. **79**, 408 (1961). R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske. J. Am. Chem. Soc. **84**, 3200 (1962). R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. V. Heyningen. J. Am. Chem. Soc. **84**, 3401 (1962).
4. G. Stork and H. T. Cheung. J. Am. Chem. Soc. **87**, 3783 (1965).
5. C. Djerassi. Steroid reactions, an outline for organic chemists. Holden-Day, Inc., San Francisco. 1963. Chap. 8.
6. J. W. ApSimon and O. E. Edwards. Can. J. Chem. **40**, 896 (1962).
7. J. F. Collins and M. H. Richmond. Nature, **195**, 142 (1962). D. Crowfoot, C. W. Bunn, B. W. Rogers-Low, and A. Turner-Jones. *In* The chemistry of penicillin. *Edited by* H. T. Clark, J. R. Johnson, and R. Robinson. Princeton University Press, Princeton, New Jersey. 1949. Chap. 11.
8. S. Wolfe and C. Ferrari. Unpublished results.
9. J. C. Sheehan. Molecular modification in drug design. Advances in Chemistry Series, No. 45, American Chemical Society, Washington, D.C. 1964. p. 15.
10. R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews. J. Am. Chem. Soc. **85**, 1896 (1963).
11. R. Pummerer. Ber. **42**, 2282 (1909); Ber. **43**, 1401 (1910).
12. E. P. Abraham and G. G. F. Newton. Biochem. J. **79**, 377 (1961).
13. D. C. Hodgkin and E. N. Maslen. Biochem. J. **79**, 393 (1961).
14. E. P. Abraham and G. G. F. Newton. *In* Ciba Foundation Symposium, amino acids and peptides with antimetabolic activity. *Edited by* G. E. W. Wolstenholme. J. A. Churchill Ltd., London. 1958. p. 205.
15. Y. Villax. Brit. Patent No. 758,653 (1956); Chem. Abstr. **51**, 6957 (1957).
16. D. A. Johnson and C. A. Panetta. J. Org. Chem. **29**, 1826 (1964).
17. F. H. Carpenter. J. Am. Chem. Soc. **70**, 2964 (1948).
18. S. Wolfe. Unpublished results.

19. S. I. LUR'E and L. G. GATSENKO. Zh. Obshch. Khim. **22**, 262 (1952).
20. J. SICHER, M. SVOBODA, and J. FARKAS. Collection Czech. Chem. Commun. **20**, 1439 (1955).
21. D. O. HOLLAND and P. MAMALIS. J. Chem. Soc. 4596 (1958).
22. A. D. HOLLEY and R. W. HOLLEY. J. Am. Chem. Soc. **72**, 2771 (1950).
23. R. B. MORIN. 148th Nat. Meeting of the American Chemical Society. Abstr. of Papers. Chicago. Sept. 1964, p. 14P; R. B. MORIN. Personal communication.
24. L. J. BELLAMY. The infrared spectra of complex molecules. Methuen and Co., Ltd., London. 1962. pp. 214–15.
25. R. B. WOODWARD. *In* The chemistry of penicillin. *Edited by* H. T. Clark, J. R. Johnson, and R. Robinson. Princeton University Press, Princeton, New Jersey. 1949. Chap. 15.
26. R. J. GILLESPIE and T. BIRCHALL. Can. J. Chem. **41**, 148 (1963).
27. R. D. COGHILL, F. H. STODOLA, and J. L. WACHTEL. *In* The chemistry of penicillin. *Edited by* H. T. Clark, J. R. Johnson, and R. Robinson. Princeton University Press, Princeton, New Jersey. 1949. Chap. 20. Y. G. PERRON, L. B. CRAST, J. M. ESSERY, R. R. FRASER, J. C. GODFREY, C. T. HOLDREGE, W. F. MINOR, M. E. NEUBERT, R. A. PARTYKA, and L. C. CHENEY. J. Med. Chem. **7**, 483 (1964). M. CLAESEN and H. VANDERHAEGHE. Bull. Soc. Chim. Belges, **73**, 647 (1964).
28. J. L. STROMINGER, J. T. PARK, and R. E. THOMPSON. J. Biol. Chem. **234**, 3263 (1959). D. J. TIPPER and J. L. STROMINGER. Proc. Nat. Acad. Sci. U.S. **54**, 1133 (1965).
29. M. L. BENDER. Chem. Rev. **60**, 53 (1960).
30. M. I. VINNIK and Y. V. MOISEYEV. Tetrahedron, **19**, 1441 (1963).
31. M. L. BENDER and R. J. THOMAS. J. Am. Chem. Soc. **83**, 4183 (1961).
32. G. C. BARRETT, V. V. KANE, and G. LOWE. J. Chem. Soc. 783 (1964).
33. A. G. LONG and A. F. TURNER. Tetrahedron Letters, 421 (1963).
34. P. L. JULIAN, E. W. MEYER, and H. C. PRINTY. J. Am. Chem. Soc. **70**, 3872 (1948).
35. W. C. J. ROSS. J. Chem. Soc. 25 (1945).
36. C. J. CAVALLITO and T. H. HASKELL. J. Am. Chem. Soc. **68**, 2332 (1946).
37. D. H. R. BARTON and C. R. NARAYANAN. J. Chem. Soc. 963 (1958).
38. C. D. HURD and K. L. KREUZ. J. Am. Chem. Soc. **72**, 5543 (1950).
39. S. GRONOWITZ and R. A. HOFFMAN. Arkiv Kemi, **15**, 499 (1960).
40. A. I. SCOTT. Interpretation of the ultraviolet spectra of natural products. Pergamon Press. New York. 1964. p. 236.
41. A. HÖRNFELDT and S. GRONOWITZ. Arkiv Kemi, **21**, 239 (1963).
42. A. H. BLATT (*Editor*). Organic syntheses. Coll. Vol. 2. John Wiley and Sons. New York. 1943. p. 570.
43. G. H. L. NEFKENS, G. I. TESSER, and R. J. F. NIVARD. Rec. Trav. Chim. **79**, 688 (1960).
44. J. C. SHEEHAN and V. J. GRENDA. J. Am. Chem. Soc. **84**, 2417 (1962).
45. R. L. SHRINER, R. C. FUSON, and D. Y. CURTIN. The systematic identification of organic compounds. 4th ed. John Wiley and Sons. London. 1959. p. 219.