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THE ANTIBACTERIAL ACTIVITY OF SOME SYNTHETIC COMPOUNDS RELATED TO PENICILLIN

BY

GEORGE BROWNLEE AND MALCOLM WOODBINE

From the Wellcome Physiological Research Laboratories, Beckenham, Kent

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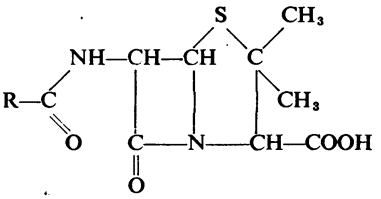
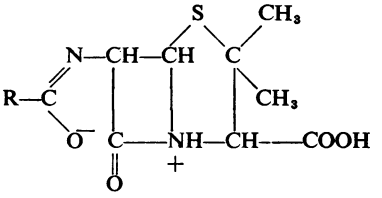
Concurrently with attempts to synthesize penicillin, the chemistry[1] and synthesis of which have been described by du Vigneaud, Carpenter, Holley, Livermore, and Rachele (1946), the antibacterial activity of compounds, or derivatives of compounds, known or postulated as parts of the penicillin molecule, has also been investigated.

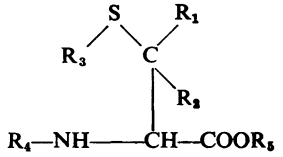
The compounds examined here may be conveniently classified into groups based on: penicillamines (I) (Copp and Wilkinson, 1947a ; Duffin and Wilkinson, 1947a, b ; Wilkinson 1947a) ; thiazolidine-4-carboxylic acids (II) (Wilkinson, 1947b) ; oxazolones (III) (Copp and Wilkinson, 1947b, c) ; derivatives of glycine (IV), and a miscellaneous group of intermediate and associated compounds (Copp, 1947) (included in Table II).



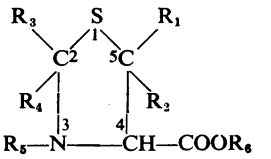
Penicillin
(with β-lactam structure)



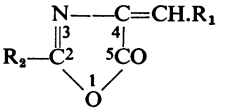
Penicillin
(with incipient azlactone structure)



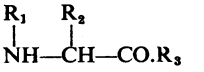
I. Penicillamines



II. Thiazolidine-4-carboxylic acids



III. Oxazolones



IV. Glycines

Results and Discussion

The antibacterial activity of the compounds, added aseptically to nutrient broth, was estimated by exposing falling concentrations of the compounds, in powers of two, to a constant inoculum of *Str. pyogenes*, CN.10. The results (Tables I and II) show that all compounds possess poor antibacterial activity *in vitro* when compared with penicillin (1,660 units per mg.). Those penicillamine esters (Table I) which possessed antibacterial activity were inactivated, partially or completely, by the presence of 10 per cent of blood or serum. The activity was not reversed by penicillinase and the morphological changes seen with penicillin were not observed.

The compounds marked T (Tables I and II) were examined for acute toxicity and for therapeutic activity in mice infected with *Str. pyogenes*, CN.10. All active compounds showed signs of toxicity within the range of 1–20 mg. per 20 g. mouse (0.5–1.0 g. per kg.), and no compound was chemotherapeutic. Loss of antibacterial activity of the esters in the presence of blood and serum suggested that the absence of chemotherapeutic action might possibly be due to hydrolysis. The

[1] Committee on Medical Research, O.S.R.D., Washington, and the Medical Research Council, London, *Nature*, 1945, **156**, 766, and *Science*, 1945, **102**, 627. The Editorial Board, Monograph on the Chemistry of Penicillin, *Science*, 1947, **105**, 653.

TABLE I

The antibacterial activity of a series of compounds expressed as units of penicillin per mg. The organism is *Streptococcus pyogenes*, CN.10. Penicillin contains 1,660 u/mg. Compounds marked T were examined for toxicity and chemotherapeutic action.

Compounds		*In vitro* activity expressed as units of penicillin per mg.			
		18 hrs.	48 hrs.	+10% blood	+10% serum
I. PENICILLAMINES					
S-Ethyl-cysteine		1/128	<1/128		
S-Ethyl-cysteine methyl ester	T	<1/128	<1/128		
Cystine *n*-butyl ester dihydrochloride		2	2		
dl-Penicillamine	T	<1/8	<1/8		
l-Penicillamine		<1/512	<1/256		
d-Penicillamine		<1/512	<1/256		
dl-Penicillamine hydrochloride	T	<1/8	<1/8		
dl-Penicillamine methyl ester hydrochloride		<1/8	<1/8		
dl-Penicillamine ethyl ester hydrochloride		1/8	1/8		
dl-Penicillamine *n*-propyl ester hydrochloride	T	1/4	1/4	1/8	1/32
dl-Penicillamine *iso*-propyl ester hydrochloride	T	1/4	1/4	1/8	1/16
dl-Penicillamine *n*-butyl ester hydrochloride	T	1/8	1/8		
dl-Penicillamine *iso*-butyl ester hydrochloride	T	1	1/4	1/4	1/8
dl-Penicillamine *n*-amyl ester hydrochloride	T	>8	16	1/4	1/8
dl-Penicillamine *iso*-amyl ester hydrochloride	T	>8	16	1/4	1/8
dl-Penicillamine *active*-amyl ester hydrochloride	T	>8	16	1/4	1/4
dl-Penicillamine *n*-hexyl ester hydrochloride	T	>8	8	1/4	1/4
dl-Penicillamine-*iso*-hexyl ester hydrochloride	T	>8	16	1/4	1/4
dl-Penicillamine benzyl ester hydrochloride	T	4	2		
N-Phenaceturyl-*dl*-penicillamine		<1/128	<1/128		
S-Benzyl-*dl*-penicillamine ethyl ester	T	1/4	2	1/28	<1/256
dl-Penicillaminic acid *n*-butyl ester hydrochloride	T	<1/128	<1/128		
N-Phenylacetyl-S-benzyl-*dl*-penicillamine methyl ester	T	<1/128	<1/128		
N-*n*-Caproyl-S-benzyl-*dl*-penicillamine methyl ester	T	<1/128	<1/128		
N-Formyl-*dl*-penicillamine methyl ester	T	<1/512	<1/512		
N-Formyl-S-benzyl-*dl*-penicillamine methyl ester	T	<1/512	<1/512		
N-Acetyl-penicillamine methyl ester	T	<1/512	<1/256		

TABLE II

The antibacterial activity of further groups of compounds against *Streptococcus pyogenes*, CN.10, expressed as units of penicillin per mg. Compounds marked T were examined for toxicity and chemotherapeutic action.

Compounds		*In vitro* activity expressed as units of penicillin per mg.		Compounds		*In vitro* activity expressed as units of penicillin per mg.	
		18 hrs.	48 hrs.			18 hrs.	48 hrs.
II. THIAZOLIDINES							
4-Carbomethoxy-2-phenyl-5 : 5-dimethyl-*dl*-thiazolidine hydrochloride	T	1/32	1/64	4-Carboxy-N-benzoyl-2 : 2 : 5 : 5-tetramethyl-*dl*-thiazolidine (ammonium salt)		<1/8	<1/8
4-Carbomethoxy-2-aminomethyl 5 : 5-dimethyl-*dl*-thiazolidine hydrochloride		<1/8	<1/8	4-Carbomethoxy-2-*spiro-cyclo*hexyl-5 : 5-dimethyl *dl*-thiazolidine hydrochloride	T	1/64	<1/32
4-Carboxy-N-phenylacetyl-2 : 2 : 5 : 5-tetramethyl-*dl*-thiazolidine (ammonium salt)		<1/8	<1/8	4-Carbo-*n*-amoxy-2-*spiro-cyclo*hexyl-5 : 5-dimethyl *dl*-thiazolidine hydrochloride	T	1/4	<1/128

TABLE II—*continued.*

Compounds	*In vitro* activity expressed as units of penicillin per mg.	
	18 hrs.	48 hrs.
II. THIAZOLIDINES—*continued.*		
4-Carbomethoxy-2-carbethoxymethyl-2 : 5 : 5-trimethyl-*dl*-thiazolidine hydrochloride T	1/32	<1/128
4-Carbomethoxy-N-benzoyl-2 : 2 : 5 : 5-tetramethyl-*dl*-thiazolidine T	1/128	<1/128
4-Carboxy-N-formyl-2 : 2 : 5 : 5-tetramethyl-*dl*-thiazolidine	<1/8	<1/8
4-Amido-N-formyl-2 : 2 : 5 : 5-tetramethyl-*dl*-thiazolidine T	<1/256	<1/256
n-Butoxy-N-formyl-2 : 2 : 5 : 5-tetramethyl-*dl*-thiazolidine T	>1	1/2
4-Carbomethoxy-N-formyl-2 : 2 : 5 : 5-tetramethyl-*dl*-thiazolidine T	<1/128	<1/128
4-Carbethoxy-N-formyl-2 : 2 : 5 : 5-tetramethyl-*dl*-thiazolidine T	<1/128	<1/128
III. OXAZOLONES		
Δ^2(4′-carboxy-5′ : 5′-dimethyl-thiazolidine)-2-phenyl-4-methyl-5-oxazolone	<1/128	<1/128
Δ^2(4′-carbomethoxy-5 : 5′-dimethyl-thiazolidine)-2-benzyl-5-oxazolone T	<1/128	<1/128
2-Phenyl-4-carbethoxymethyl-aminomethylene-5-oxazolone T	<1/512	<1/256
2-Phenyl-4-benzylthiomethylene-5-oxazolone T	<1/512	<1/256
2-Phenyl-4-(4′-aminobenzene-sulphonamido)-methylene-5-oxazolone	<1/128	<1/128
2-Phenyl-4-(2′-carboxyanilino)-methylene-5-oxazolone	<1/128	<1/128
2-Phenyl-4-(4′-aminoanilino)-methylene-5-oxazolone	<1/128	<1/128
2-Phenyl-4-(4′-amino-4′-diphenyl-aminomethylene)-5-oxazolone	<1/128	<1/128
2-Phenyl-4-(4′-carbethoxy-anilinomethylene)-5-oxazolone	<1/128	<1/128
2-Phenyl-4-(3′-aminoanilino-methylene)-5-oxazolone	<1/128	<1/128
2-Phenyl-4-(ethoxymethylene)-5-oxazolone	<1/8	<1/8
2-Phenyl-4-(ethylthiomethylene)-5-oxazolone T	<1/64	<1/128
2-Phenyl-4-(benzylthiomethylene)-5-oxazolone T	<1/512	<1/512
IV. GLYCINE ESTERS		
Glycine methyl ester hydrochloride T	<1/32	<1/32
Glycine ethyl ester hydrochloride T	<1/32	<1/32
Glycine *n*-propyl ester hydrochloride T	<1/32	<1/32
Glycine *n*-butyl ester hydrochloride T	<1/32	<1/32
Glycine *iso*-butyl ester hydrochloride T	<1/32	<1/32
Glycine *n*-amyl ester hydrochloride T	<1/32	<1/32
Triglycylglycine *n*-butyl ester T	<1/32	<1/32
Phenylglycine T	<1/512	<1/512
N-Formyl-glycine methyl ester T	<1/512	<1/512
N-Formyl-glycinamide T	<1/512	<1/512
α-Formyl-N-benzoyl-glycine ethylthioester T	1/128	1/256
N-Benzoyl-α-ethylthiomethylene-glycinebenzylthio ester T	<1/512	<1/512
N-Methyl-glycine ethyl ester hydrochloride (Sarcosine ethyl ester) T	<1/512	<1/256
N-Benzylglycine ethyl ester hydrochloride T	<1/512	<1/256
N′-(α-Formyl-N-benzoylglycyl)-*p*-aminobenzenesulphonamide T	1/256	1/128
N′-(N-Benzoyl-α-ethylthio-methylene-glycyl)-*p*-aminobenzene-sulphonamide T	1	1/8
N′(N-Benzoyl-α-benzylthiomethylene-glycyl)*p*-aminobenzenesulphon-amide T	1/16	1/8
MISCELLANEOUS		
n-Butyl phenaceturate	1/128	<1/128
n-Amyl phenaceturate	1/64	1/128
Ethyl α-amino-ββ-dimethylacrylate T	1/32	1/16
Methyl α-benzamido-ββ-dimethyl-acrylate T	<1/32	<1/16
Ethyl α-(N-benzylbenzamido)-β-hydroxyacrylate T	<1/512	<1/256
Ethyl α-(N-methylacetamido)-β-benzylaminoacrylate T	1/512	1/512
Ethyl α-(N-methylbenzamido)-β-hydroxyacrylate T	<1/512	<1/512
Mandelylalanine T	<1/512	<1/512
Acetylmandelylalanine	<1/512	<1/512
Phenylaminoacetylalanine T	<1/512	<1/512
N-Δ^{α}-Hexenoylalanine (ammonium salt) T	<1/512	<1/512
Hippurylamide T	<1/512	<1/512
Ethyl-N-benzyl hippurate T	1/512	<1/512
N-Benzylhippuric hydrazide T	1/512	<1/512
5-Carbomethoxy-2-phenyl-tetrahydro-1 : 4-thiazone-3 T	1/32	1/16
5-Carboxy-2-phenyltetrahydro-1 : 4-thiazone-3 (ammonium salt) T	<1/32	<1/16
bis-Phenylchloracetyl-cystine dimethyl ester T	<1/32	<1/16
Benzyl mercaptan T	<1/32	<1/16
Tribenzylthiocarbinol T	<1/512	<1/512
2-Benzoylamino-3-pyrazolone T	1/128	<1/128
Methyl *nor*penicillenate T	<1/512	<1/512
Sodium *nor*penicillenate T	<1/512	<1/512
3-Keto-4-carbethoxy-4-phenyl-Δ^{α}-pentenoic acid T	1/128	1/32
Formamide T	<1/128	<1/128
dl-Valine *n*-butylester hydrochloride T	1/128	1/256

observation that mice given toxic doses of *dl*-penicillamine-*n*-butyl ester hydrochloride by the intraperitoneal route were first anaesthetized and only later succumbed to the convulsions characteristic of penicillamine added verisimilitude to this possibility. Comparisons of the acute toxicity of *dl*-penicillamine-*n*-butyl ester hydrochloride and its constituents were made in groups of ten mice. The results (Table III) suggest that, when due allowance is made for the rate of hydrolysis, the acute toxicity of the compound approximates to that of the mixture of its constituent parts.

TABLE III

The average lethal doses of *dl*-penicillamine-*n*-butyl ester hydrochloride and its constituents when administered intraperitoneally to groups of 10 mice.

Compound	LD50 ± SD (mg. per 20 g.)
dl-Penicillamine hydrochloride	4.0 ± 0.6
dl-Penicillamine hydrochloride + *n*-butyl alcohol	10.5 ± 1.3
dl-Penicillamine-*n*-butyl ester hydrochloride	13.0 ± 1.6
n-Butyl alcohol	35.0 ± 5.7

Summary

1. Some penicillamine, thiazolidine, oxazolone, glycine, and associated compounds have been examined for chemotherapeutic activity.

2. The esters of penicillamine possess antibacterial activity *in vitro*, but their mode of action is not related to that of penicillin. They are inactivated in the presence of blood or serum, and evidence is presented which indicates that this may be due to hydrolysis.

3. The more active compounds, when administered by the intraperitoneal route, were acutely toxic in small doses to mice.

4. None of the compounds possesses chemotherapeutic value.

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References

Copp, F. C. (1947). *British Patent*, 592,461.

Copp, F. C., and Wilkinson, S. (1947a). *British Patent*, 585,250.

Copp, F. C., and Wilkinson, S. (1947b). *British Patent*, 588,960.

Copp, F. C., and Wilkinson, S. (1947c). *British Patent*, 589,406.

Duffin, W. M., and Wilkinson, S. (1947a). *British Patent*, 585,413.

Duffin, W. M., and Wilkinson, S. (1947b). *British Patent*, 585,436.

Du Vigneaud, V., Carpenter, F. H., Holley, R. W., Livermore, A. H., and Rachele, J. R. (1946). *Science*, **104**, 431.

Wilkinson, S. (1947a). *British Patent*, 585,439.

Wilkinson, S. (1947b). *British Patent*, 593,522.