Journal of Medicinal and Pharmaceutical Chemistry

VOL. 4, No. 1 (1961)

Note

Synthesis of Penicillins Using Dicyclohexylcarbodiimide as a Condensing Agent

DONALD C. HOBBS and ARTHUR R. ENGLISH, *Biochemical Research and Development Department, Chas. Pfizer & Co., Inc., Groton, Conn.* and *Maywood, N.J.*

The availability of APA,* the penicillin nucleus,[1] has made possible the preparation of new penicillins not previously available by fermentation procedures. The treatment of APA with the acid chloride or anhydride of the appropriate acid has been used to prepare such penicillins.[2] In order to prepare for biological testing as wide a range of new penicillins as possible, alternate methods for the condensation of carboxylic acids with APA were investigated. The use of DCCI* as a condensing agent[3] met the requirements of ease of preparation, ability to use small quantities of acid, and freedom from interfering side-products.

To test the generality of the method and to assess the effect of various functional groups on biological activity, a group of acids, selected for their diversity of structure, were condensed with APA. A mixture of 1 ml of a 10 mg/ml solution of DCCI in tetrahydrofuran (THF), 1 ml of a 10 mg/ml solution (or suspension) of the acid in THF, and 1 ml of the sodium salt of APA in H_2O–THF (1 : 1) was shaken for 2 h at room temperature. After dilution with water and filtration (to remove dicyclohexylurea and unreacted DCCI), the solution was sterilized by passage through a sintered glass filter. Minimum inhibitory concentrations of test organisms other than *Mycobacterium 607* were determined by the commonly accepted two-fold serial dilution technique in brain–heart infusion medium (Difco). Nutrient broth adjuncted with 10 per cent glycerol was used for *Mycobacterium 607*. Inocula were taken from 1 : 1000 dilution of 18 h cultures grown at 37°;

* The abbreviations used are: APA, 6-aminopenicillanic acid; DCCI, *N,N'*-dicyclohexylcarbodiimide.

test incubation was for 18 to 20 h at 37°. The lowest concentration which prevented visible growth during this incubation period was taken as the minimum inhibitory concentration.

Treatment of a portion of the reaction mixture with penicillinase,* followed by paper chromatography and quantitative ninhydrin determination, was used to estimate penicilloic acid (from unreacted APA) and thus indicate the extent of condensation. Values varied widely with the acid employed and were used to correct the preliminary biological data. The extent of condensation for the various acids is indicated in Table I.

Paper chromatography of the penicillins indicated, by low R_f values, that polar groups when present in the acids were also present in the penicillins. Duplicate sheets, sprayed with reagents sensitive to mercapto, amino and nitro groups, indicated the presence of those groups in areas corresponding to the biologically active zones of the appropriate penicillins. The previously known penicillins had R_f values on several systems identical to those of authentic samples. Minor components were noted on occasion.

Where the initial screening indicated interest in a penicillin, either by high activity or by an alteration in the antibacterial spectrum, the above procedure, on a larger scale, was used to prepare sufficient material for further testing. The reaction mixture was acidified and extracted with butanol and the latter extracted with water at pH 7.† The aqueous solution of the penicillin was lyophilized and the solid used for *in vitro* and *in vivo* testing. The purity of this solid preparation was directly determined by the hydroxylamine[4] procedure; the results of this assay are given in the Table. The *in vitro* minimum inhibitory concentrations against five representative organisms are also included in the Table.

The broad areas of greatest interest, determined in the above manner, are being intensively pursued and will be the subject of future publications.

(*Revised manuscript received 12 January, 1961*)

* Baltimore Biological Laboratory, Inc.

† The diluted reaction mixture, in the case of valine, was extracted with ether and lyophilized directly.

Table I. Yield, purity and microbiological activity of penicillins prepared using dicyclohexylcarbodiimide

	% reacted	Purity, %	Minimum inhibitory concentrations[a]				
			Staph. aureus 5[b]	*Staph. aureus 400*[c]	*E. coli*	*Salmonella typhosa*	*Mycobacterium 607*
Phenylacetic (Penicillin G)	71	46	0·045	>100	25	12·5	25
Mandelic	70	42	1·56	>100	100	50	—
o-Mercaptobenzoic	48	16	6·25	25	50	100	50
Coumarilic	56	38	3·12	>100	>100	>100	0·39
Valine	57	10	12·5	25	12·5	1·56	3·12
N-Acetyl-α-aminobutyric	70	33	3·12	>100	100	50	100
α-Methoxyphenylacetic	73	22	0·19	>100	50	50	50
Allylmercaptoacetic (Penicillin O)	30	70	0·03	>100	50	12·5	50
3-Hexenoic (Penicillin F)	30	17	0·09	>100	100	1·56	25
p-Nitrobenzoic	57	10	0·39	>100	50	12·5	25
Adipic[d]	87	35	1·56	>100	>100	6·25	>100
α-Bromobutyric	52	43	0·19	>100	>100	>100	0·19
4-Cyanobenzoic	63	17	0·78	>100	100	50	>100
Epoxysuccinic	73	71	12·5	>100	100	100	0·50
Monomethylsuccinate	47	30	0·78	200	25	25	100
Acetylmercaptoacetic	45	90	0·78	>100	100	25	—
Cyclobutanecarboxylic	54	15	0·19	>100	>100	>100	100
β-Ketoglutaric	63	10	1·56	>100	25	3·12	>100

[a] Micrograms per ml. Values were corrected for purity of the penicillin.
[b] Penicillin-sensitive strain.
[c] Penicillin-resistant strain.
[d] Described by Ballio *et al.* *Nature, Lond.*, **185**, 97, 1960.

References

[1] Batchelor, T., Doyle, F., Nayler, J. H. C. and Rolinson, G. *Nature, Lond.*, **183**, 257 (1959)
[2] Perron, Y. G., Minor, W. F., Holdredge, C. T., Gottstein, W. J., Godfrey, J. C., Crast, L. B., Babel, R. B. and Cheney, L. C. *J. Amer. chem. Soc.*, **82**, 3934 (1960)
[3] Sheehan, J. C. and Hess, G. P. *J. Amer. chem. Soc.*, **77**, 1067 (1955)
[4] Ford, J. H. *Analyt. Chem.*, **19**, 1004 (1947)