JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

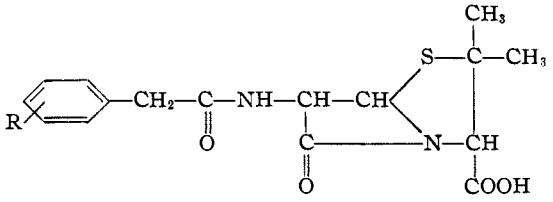
VOLUME 70 SEPTEMBER 30, 1948 NUMBER 9

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Biosynthesis of Penicillins. V.[1] Substituted Phenylacetic Acid Derivatives as Penicillin Precursors

BY JOSEPH W. CORSE,[2] REUBEN G. JONES, QUENTIN F. SOPER, CALVERT W. WHITEHEAD AND OTTO K. BEHRENS

In the previous papers of this series it has been shown that certain precursor substances when added to the culture media may profoundly influence both the quantity and kind of penicillins formed by penicillin-producing molds.[1] It has been established that the mold is able to incorporate the phenylacetyl portion of N-phenylacetyl-DL-valine into benzylpenicillin (Penicillin G).[3] With these facts in mind it was of interest to determine whether phenylacetic acid derivatives containing substituents, R, on the benzene nucleus also could be utilized by the mold to form new penicillins (I). That such new penicillins are



formed, frequently to the exclusion of other penicillins, has been demonstrated. The preceding paper[1] of this series described the isolation and characterization of a number of these new penicillins.

The purpose of this communication is to present the series of substituted phenylacetic acid derivatives which have been tested as precursor substances. In Table I are listed the DL-valine derivatives, and in Table II the N-2-hydroxyethyl amides. The valine and 2-hydroxyethylamine compounds were selected on the basis of earlier work with a large number of phenylacetic acid derivatives (penicillin G precursors).[4] These earlier experiments indicate that N-phenylacetyl-DL-valine and N-2-hydroxyethylphenylacetamide were among those precursors best utilized by the mold.

Also included in this paper is a series of derivatives of dithiophenylacetic acid. These compounds are the amides obtained from the acid and DL-valine, D- and L-penicillamine, DL-isoleucine, β,β-diethoxyalanine and methyl-*n*-propylamine. No significant stimulation was obtained from any of these products.

In the last two columns of Tables I and II are recorded the results of tests carried out in shake flask cultures using *P. notatum*, strain NRRL 1976. A number of the compounds in Tables I and II were also tested with *P. chrysogenum* Q176 and in almost all cases the results were similar. The methods of carrying out these tests and the interpretation and significance of the results have been fully discussed elsewhere.[5] In general, an increase in penicillin yield indicates that a new penicillin has been formed. Additional evidence for the presence or absence of a new penicillin has been obtained in a number of cases by partition of the penicillin mixture in the Craig machine.[5] However, final proof of the existence of a new penicillin rests with its isolation and characterization.[1]

One of the acids of this group, namely, *p*-hydroxyphenylacetic acid, is the precursor of the naturally-occurring *p*-hydroxybenzylpenicillin

(1) For the preceding paper of this series see: Behrens, Corse, Edwards, Garrison, Jones, Soper, Van Abeele and Whitehead, *J. Biol. Chem.*, **175**, 793 (1948).

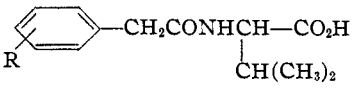
(2) Present address, University of California at Los Angeles, Los Angeles, California.

(3) Behrens, Corse, Jones, Kleiderer, Soper, Van Abeele, Larson, Sylvester, Haines and Carter, *J. Biol. Chem.*, **175**, 765 (1948).

(4) Behrens, Corse, Jones, Mann, Soper, Van Abeele and Chiang, *ibid.*, **175**, 751 (1948).

(5) Behrens, Corse, Huff, Jones, Soper and Whitehead, *ibid.*, **175**, 771 (1948).

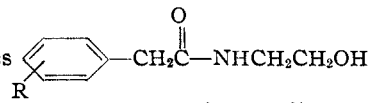
TABLE I

SUBSTITUTED PHENYLACETYL-DL-VALINE DERIVATIVES 

R[a]	Solvent[b]	M. p., °C.	Formula	Analyses, % N Calcd.	Found	Stimulation[c]
o-Amino[d]	A	238–241	$C_{13}H_{18}N_2O_3$	11.19	11.29	1.37
p-Amino[d]	A	220–227	$C_{13}H_{18}N_2O_3$	11.19	11.21	1.0
p-Benzyloxy	A	144–145	$C_{20}H_{23}NO_4$	4.10	4.19	1.0
p-Carbethoxyhydroxy	B	125–127	$C_{16}H_{21}NO_6$	4.33	4.50	1.0
o-Chloro[6]	C	122–124	$C_{13}H_{16}ClNO_3$	5.19	4.80	1.0
p-Chloro[7]	D	144–145	$C_{13}H_{16}ClNO_3$	5.19	5.00	1.33
p-Cyano[8]	A	138–140	$C_{14}H_{16}N_2O_3$	10.76	10.89	1.24
p-Iodo[9]	A	148–150	$C_{13}H_{18}INO_3$	3.88	3.94	1.0
p-Isopropyl[10]	E	114–115	$C_{16}H_{23}NO_3$	5.04	4.84	1.0
p-Methoxy[11]	F	129	$C_{14}H_{19}NO_4$	5.27	5.21	1.52
o-Nitro[12]	C	173–175	$C_{13}H_{16}N_2O_5$	10.00	9.77	1.0
m-Nitro[13]	A	153–158	$C_{13}H_{18}N_2O_5$	10.00	10.10	0.88
p-Nitro[14]	D	134–135	$C_{13}H_{16}N_2O_5$	10.00	10.15	1.49
p-Nitro[e]	D	113–115	$C_{14}H_{18}N_2O_5$	9.65	9.65	1.29
2,4,6-Trimethyl[15]	A	130–132	$C_{16}H_{23}NO_3$	5.04	4.87	1.0

[a] References indicate the procedure in literature which has been followed for the preparation of the acid. [b] Solvents used for recrystallization were: A. ethanol–ether–petroleum ether, B. ethanol–ether, C. methanol, D. ethanol, E. ether–petroleum ether, F. methyl acetate–petroleum ether. [c] Compounds were tested at 0.0008 molar concentration. The value recorded represents the ratio: units in test container/units in control container. We acknowledge with thanks the numerous assays performed by Dr. J. M. McGuire. [d] Prepared by catalytic reduction of the corresponding nitro compound with Adams catalyst. [e] Isoleucine derivative.

TABLE II

N-(2-HYDROXYETHYL) SUBSTITUTED PHENYLACETAMIDES 

R[a]	M. p., °C.	Formula	Analyses, % N Calcd.	Found	Stimulation[b]
p-Acetylamino[18]	145–146	$C_{12}H_{17}N_2O_3$	11.81	11.86	1.0
p-Allyloxy	84–85	$C_{13}H_{17}NO_3$	5.94	6.28	1.23
4-Amino-3-nitro	132	$C_{10}H_{13}N_3O_4$	[c]		1.0
p-Amino	103–104	$C_{10}H_{15}N_2O_2$	14.42	14.56	1.14
p-*t*-Amyl	[d]	$C_{15}H_{22}NO_2$	5.61	5.58	1.0
p-Anisoylamino	210–211	$C_{18}H_{20}N_2O_4$	[e]		1.0
4-Bromo-3-chloro	104–106	$C_{10}H_{11}BrClNO_2$	4.79	4.95	1.71
o-Bromo[17]	106–107	$C_{10}H_{12}BrNO_2$	5.43	5.51	1.0
m-Bromo[18]	129–130	$C_{10}H_{12}BrNO_2$	5.43	5.37	2.21
p-Bromo[19]	108–109	$C_{10}H_{12}BrNO_2$	5.43	5.40	2.90
p-*t*-Butyl	[d]	$C_{15}H_{22}NO_2$	5.61	5.58	1.0
o-Chloro[6]	99–100	$C_{10}H_{12}ClNO_2$	6.55	6.43	1.0
m-Chloro[20]	114–117	$C_{10}H_{12}ClNO_2$	6.55	6.54	1.84
p-Chloro[7]	90–91	$C_{10}H_{12}ClNO_2$	6.55	6.48	1.97
3,5-Diacetomercuri-4-hydroxy	.....	$C_{14}H_{17}Hg_2NO_7$	1.97[f]	2.10	1.0
3,5-Dibromo-4-hydroxy[9]	200–202	$C_{10}H_{11}Br_2NO_3$	3.97	3.66	1.0
3,4-Dibromo	125–127	$C_{10}H_{11}Br_2NO_2$	4.16	4.22	1.0
2,4-Dichloro[h]	118–119	$C_{10}H_{11}Cl_2NO_2$	5.64	5.71	1.0
3,4-Dichloro[h]	113–114	$C_{10}H_{11}Cl_2NO_2$	5.64	5.79	2.10
p-(γ-Diethylaminopropoxy)	[d]	$C_{17}H_{28}N_2O_3$	9.08	8.86	1.0
p-(β,γ-Dihydroxypropoxy)	[d]	$C_{13}H_{19}NO_5$	5.20	5.31	1.20
3,5-Diiodo-4-hydroxy[i]	179–180	$C_{10}H_{11}NI_2O_3$	3.12	3.03	1.0

(6) Mehner, *J. prakt. Chem.*, **62**, 554 (1900).
(7) Straus, *Ann.*, **393**, 317 (1912).
(8) Jaeger and Robinson, *J. Chem. Soc.*, 745 (1941).
(9) Datta and Chatterjee, THIS JOURNAL, **41**, 295 (1919).
(10) Rossi, *Ann. suppl.*, **1**, 139 (1861).
(11) Cain, Simonsen and Smith, *J. Chem. Soc.*, **103**, 1036 (1913).
(12) Reissert, *Ber.*, **30**, 1041 (1897); *ibid.*, **41**, 3814, 3925 (1908).
(13) Gabriel and Borgmann, *Ber.*, **16**, 2064 (1883).
(14) Robertson, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 406.
(15) The mesitylacetic acid used was kindly furnished by Dr. R. C. Fuson of the University of Illinois, *cf.* "Organic Syntheses," **25**, 65 (1945).
(16) Ferber and Bendix, *Ber.*, **72**, 839 (1939).
(17) Shuttleworth, Rapson and Stewart, *J. Chem. Soc.*, 73 (1944).
(18) Berger, *J. prakt. Chem.*, **133**, 331 (1932).
(19) Wislicenus and Grützner, *Ber.*, **42**, 1933 (1909).
(20) Kenner and Morton, *J. Chem. Soc.*, 679 (1934).

TABLE II (*Continued*)

R[a]	M. p., °C.	Formula	Analyses, % N Calcd.	Found	Stimulation[b]
2,3-Dimethoxy[21]	93	$C_{12}H_{17}NO_4$	5.85	5.83	1.0
3,4-Dimethoxy[21]	96–98	$C_{12}H_{17}NO_4$	5.85	5.81	1.0
3,4-Dimethyl	99–100	$C_{12}H_{17}NO_2$	6.75	7.03	1.27
p-Ethoxy[22]	90–91	$C_{12}H_{17}NO_3$	6.27	6.28	1.26
o-Fluoro	103–105	$C_{10}H_{12}FNO_2$	7.10	7.09	1.23
m-Fluoro	75–77	$C_{10}H_{12}FNO_2$	7.10	7.10	1.93
p-Fluoro	75	$C_{10}H_{12}FNO_2$	7.10	7.03	1.54
o-Hydroxy[j]	[d]	$C_{10}H_{13}NO_3$	7.17	7.39	1.24
m-Hydroxy[k]	92–93	$C_{10}H_{13}NO_3$	7.17	7.58	1.13
p-Hydroxy[23]	110–112	$C_{10}H_{13}NO_3$	7.17	7.18	1.0
p-Hydroxy[l]	84–86	$C_{11}H_{13}NO_2$	7.33	7.19	1.0
p-(N-2-Hydroxyethylcarbamyl)[24]	157–158	$C_{13}H_{18}N_2O_4$	[m]		1.0
4-Hydroxy-3-phenylazo	180–181.5	$C_{16}H_{17}N_3O_3$	14.04[n]	14.43	1.0
m-Iodo	127–129	$C_{10}H_{12}INO_2$	4.59	4.93	1.75
p-Iodo[9]	112–113	$C_{10}H_{12}INO_2$	4.59[o]	4.32	1.83
5-Isopropyl-2-methyl[25]	[d]	$C_{14}H_{21}NO_2$	5.95	6.20	1.0
p-Isopropyl[10,24]	[d]	$C_{13}H_{19}NO_2$	6.33	6.18	1.33
o-Methoxy[p]	[d]	$C_{11}H_{15}NO_3$	6.69	6.06	1.0
m-Methoxy[26]	59	$C_{11}H_{15}NO_3$	6.69	6.68	1.0
p-Methoxy[q,r]	86–88	$C_{11}H_{15}NO_3$	6.69	6.64	1.22
3,4-Methylenedioxy[p]	99–100	$C_{11}H_{13}NO_4$	6.28	6.42	1.0
p-Methylmercapto	115–117	$C_{11}H_{15}NO_2S$	6.30	6.30	1.49
4-Methoxy-3-nitro	69	$C_{11}H_{14}N_2O_5$	11.02	10.81	1.0
o-Methyl[27]	63–64	$C_{11}H_{15}NO_2$	7.24	7.16	1.36
m-Methyl[28]	[d]	$C_{11}H_{15}NO_2$	7.24	7.21	1.39
p-Methyl[29]	76–78	$C_{11}H_{15}NO_2$	7.24	7.28	1.69
p-Nitro[14,30]	140–142	$C_{10}H_{12}N_2O_4$	12.50	12.54	1.0
p-Phenoxy	95	$C_{16}H_{17}NO_3$	5.16	5.24	1.64
p-Phenylmercapto	89–90	$C_{16}H_{17}NO_2S$	4.93	4.97	1.0
p-Phenyl[29]	172–175	$C_{15}H_{17}NO_2$	5.75	5.70	0.87
3,4,5-Tribromo	212–213	$C_{10}H_{10}Br_3NO_2$	3.37	3.39	0.33
m-Trifluoromethyl	[d]	$C_{11}H_{12}F_3NO_2$	[s]		1.28
2,4,6-Trimethyl[31]	144–145	$C_{13}H_{19}NO_2$	6.33[t]	6.60	1.0

[a] References are to the phenylacetic acids or their esters (*cf.* ref. *a*, Table I). [b] See explanation, footnote *c*, Table I. [c] Calcd.: C, 50.21; H, 5.48. Found: C, 50.51; H, 5.71. [d] Oil. [e] Calcd.: C, 54.56; H, 4.58. Found: C, 54.55; H, 4.58. [f] Calcd.: C, 23.60; H, 2.41. Found: C, 23.84; H, 2.47. [g] Prepared by the bromination of N-(2-hydroxyethyl)-*p*-hydroxyphenylacetamide in aqueous solution with a potassium bromide–bromine mixture. [h] Prepared from the dichlorobenzyl halides (see Experimental). No intermediates were isolated. [i] Prepared by iodination of N-(2-hydroxyethyl)-*p*-hydroxyphenylacetamide with iodine monochloride in glacial acetic acid. [j] The ethyl ester was obtained from methyl *o*-methoxyphenylacetate by hydrolysis first with aqueous–ethanolic potassium hydroxide, ether cleavage by refluxing overnight with hydriodic acid (d. 1.7) and finally esterification with ethanol and sulfuric acid. The ester boiled at 96–100° (0.2 mm.). [k] Made from methyl *m*-methoxyphenylacetate as described under *j* for the *o*-isomer. [l] Allyl amide. [m] Calcd.: C, 58.63; H, 6.81. Found: C, 58.48; H, 6.76. [n] Calcd.: C, 64.20; H, 5.74. Found: C, 63.22; H, 5.50. [o] Calcd.: C, 39.36; H, 3.97. Found: C, 39.32; H, 4.01. [p] The procedure of ref. 21 was used. [q] The procedure of ref. 21 was used, *cf.* Mauthner, *Ann.*, **370**, 374 (1909). [r] N-2-Aminoethyl-*p*-methoxyphenylacetamide hydrochloride was prepared from the ester and ethylenediamine by the method of Aspinall, THIS JOURNAL, **63**, 852 (1941), m. p. 135–138°. Calcd. for $C_{11}H_{16}N_2O_2 \cdot HCl$: N, 11.48. Found: N, 11.26. Stimulation 1.34. [s] Calcd.: C, 53.44; H, 4.89. Found: C, 53.44; H, 5.28. [t] Calcd.: C, 70.56; H, 8.65. Found: C, 69.73; H, 8.39.

(21) Snyder, Buck and Ide, "Organic Syntheses," Coll. Vol. II, 333 (1943). From 3,4-dimethoxybenzaldehyde there was obtained methyl 3,4-dimethoxyphenylacetate, b. p. 180–183° (30 mm.).

(22) Werner, *Ann.*, **322**, 148 (1902).

(23) Salkowski, *Ber.*, **22**, 2140 (1889).

(24) Fileti and Basso, *Gazz. chim. ital.*, **21**, I, 52 (1891).

(25) Willgerodt, *J. prakt. Chem.*, [2] **80**, 184 (1909). Modified according to Schwenk and Papa, *J. Org. Chem.*, **11**, 798 (1946), and hydrolyzed with 2:1 hydrochloric acid. Methyl 5-isopropyl-2-methylphenylacetate boiled at 94° (1 mm.).

(26) Pschorr, *Ann.*, **391**, 44 (1912).

(27) Radiszewski and Wisper, *Ber.*, **18**, 1281 (1885).

(28) Senkowski, *Monatsh.*, **9**, 855 (1888).

(29) Willgerodt and Scholtz, *J. prakt. Chem.*, [2] **81**, 382 (1910), as modified ref. 25.

(30) Maxwell, *Ber.*, **12**, 1765 (1879).

(31) Meyer and Sudborough, *Ber.*, **27**, 1587 (1894).

(Penicillin X). Under the conditions of the experiments, *p*-hydroxyphenylacetic acid and its derivatives (Tables I and II) gave no stimulation in penicillin yield when added to the culture media. Nevertheless it was possible to demonstrate that *p*-hydroxybenzylpenicillin constituted at least 8% of the crude penicillin produced by *P. notatum* (NRRL 1976) grown in the presence of N-(2-hydroxyethyl)-*p*-hydroxyphenylacetamide. In the absence of *p*-hydroxyphenylacetic acid derivatives this strain produced no detectable quantity of *p*-hydroxybenzylpenicillin.

In some cases in which there was no significant stimulation, and the formation of a new penicillin

was thus in doubt, recourse was had to the Craig apparatus for resolving the mixtures. With our conditions of testing, this technique failed to show that significant quantities of new penicillins were formed from the N-2-hydroxyethyl amides of *p*-acetylaminophenylacetic, *m*-hydroxyphenylacetic, *p*-(β,γ-dihydroxypropoxy)-phenylacetic, and 3,5-diacetomercuri-4-hydroxyphenylacetic acids.

From the results of the stimulation data (Tables I and II) it is difficult to draw any generalizations. Both the kind and position of the substituents had a marked influence upon the ability of the compound to act as a penicillin precursor. That the nature of the phenylacetic acid derivative had a profound influence upon its utilization by the mold was illustrated in several cases. For example, *p*-nitrobenzylpenicillin was produced in good yield when *p*-nitrophenylacetylvaline was used as precursor, whereas N-2-hydroxyethyl-*p*-nitrophenylacetamide gave no stimulation in yield. On the other hand, N-2-hydroxyethyl-*p*-iodophenylacetamide gave a high stimulation whereas *p*-iodophenylacetylvaline gave none.

For the most part the compounds of Tables I and II were prepared by well-known methods, details of which are included in the Experimental section.

Experimental

Valine Derivatives.—Preparation of the valine derivatives (Table I) is illustrated by the synthesis of N-(*p*-benzyloxyphenylacetyl)-DL-valine.

***p*-Benzyloxyphenylacetic Acid.**—Ethyl *p*-hydroxyphenylacetate[28] (36 g.) was added to 300 ml. of absolute ethanol containing 13.3 g. of sodium methoxide. Then 38 g. of benzyl chloride was added and the resulting mixture was boiled under reflux with stirring overnight. The alcohol was removed by distillation and the residue was dissolved in ether. The ether solution was washed with dilute sodium hydroxide solution and the ether removed *in vacuo*. The residue was mixed with 400 ml. of ethanol, 70 g. of potassium hydroxide and 70 ml. of water and heated under reflux overnight. The alcohol was removed and the residue was dissolved in water. Acidification yielded 15.2 g. of acid after recrystallization from a mixture of benzene and petroleum ether. It melted at 120–121°.

Anal. Calcd. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.45. Found: C, 74.60; H, 5.61.

N-(*p*-Benzyloxyphenylacetyl)-DL-valine.—The above acid was dissolved in 30 ml. of thionyl chloride and the mixture was allowed to stand overnight. After the excess thionyl chloride had been removed at reduced pressure, a Schotten–Baumann reaction was run with the residue on 11.7 g. of DL-valine using 16 ml. of 12 *N* sodium hydroxide solution and 200 ml. of water. The product was recrystallized from a mixture of ethanol, ether and petroleum ether.

***p*-Carbethoxyhydroxyphenylacetic Acid.**—A solution of 15.2 g. of *p*-hydroxyphenylacetic acid in 200 ml. of water to which 48.4 ml. of 4.135 *N* sodium hydroxide solution had been added, was stirred in an ice-bath, and 12 g. of ethyl chlorocarbonate was added dropwise. After the mixture had been stirred for two hours, 32 ml. of 4 *N* hydrochloric acid was added and the precipitate was collected on a filter. After recrystallization from ether–petroleum ether, the acid melted at 78–79°.

Anal. Calcd. for $C_{11}H_{12}O_5$: C, 58.92; H, 5.32. Found: C, 58.90; H, 5.37.

N-2-Hydroxyethyl Amides.—These amides (Table II) were prepared by heating the esters of the substituted phenylacetic acids with an excess of ethanolamine overnight on the steam-bath or at 110–120° for several hours. The excess amine was removed by evaporation *in vacuo* and the residue recrystallized from ethylene dichloride. Occasionally mixtures of methanol–ethyl acetate or ethanol–ether–petroleum ether were used for recrystallization.

Methyl 3,4-Dibromophenylacetate.—3,4-Dibromotoluene was prepared by the method of Fieser and Bowen[32] from 185 g. of 4-amino-3-bromotoluene. The yield was 98 g., b. p. 113° (6 mm.).

Ninety-eight grams of the dibromotoluene was placed in a 250-ml. flask equipped with a reflux condenser and a gas inlet tube extending almost to the bottom. The mixture was heated to boiling and then 63 g. of bromine was swept into the flask by means of a stream of warm air; this process took three hours. A mixture of 122 g. of the resulting crude 3,4-dibromobenzyl bromide, 38 g. of potassium cyanide, 75 ml. of water and 225 ml. of ethanol was boiled under reflux for four hours. The alcohol was then removed by distillation and the inorganic salts by filtration. The resulting oil was dissolved in ether and the solution was washed with dilute alkali and with dilute sodium bisulfite solution. The ether was removed on a steam-bath. The crude nitrile was hydrolyzed by refluxing for six hours in a solution of 75 ml. of concentrated sulfuric acid, 100 ml. of water and 300 ml. of ethanol. The alcohol was then removed by distillation and the crude acid separated. It was partially purified by dissolving in sodium carbonate solution followed by precipitation with hydrochloric acid. This crude 3,4-dibromophenylacetic acid was esterified with methanol and sulfuric acid to give methyl 3,4-dibromophenylacetate, m. p. 44–45°, from ethanol.

Anal. Calcd. for $C_9H_8Br_2O_2$: C, 35.10; H, 2.62. Found: C, 34.93; H, 2.52.

The following esters were prepared from the corresponding toluenes in a manner similar to that just described.

Methyl 3,4,5-tribromophenylacetate, m. p. 78–79°, was obtained from 3,4,5-tribromotoluene[33] without isolating any intermediate.

Anal. Calcd. for $C_9H_7Br_3O_2$: C, 27.94; H, 1.82. Found: C, 27.13, 27.20; H, 1.65, 1.78.

Methyl 4-bromo-3-chlorophenylacetate, m. p. 42–43° was prepared from 4-amino-3-chlorotoluene.[32]

Anal. Calcd. for $C_9H_8BrClO_2$: C, 41.02; H, 3.06. Found: C, 40.75; H, 2.89.

Ethyl *o*-fluorophenylacetate, b. p. 123–125° (24 mm.), was obtained in a 52% over-all yield from *o*-fluorotoluene.

Anal. Calcd. for $C_{10}H_{11}FO_2$: C, 65.92; H, 6.08. Found: C, 66.01; H, 6.21.

Ethyl *m*-fluorophenylacetate, b. p. 126–129° (28 mm.), 85–90° (1 mm.), was obtained from *m*-fluorotoluene in 22% yield.

Anal. Calcd. for $C_{10}H_{11}FO_2$: C, 65.92; H, 6.08. Found: C, 65.80; H, 6.61.

Ethyl *p*-fluorophenylacetate, b. p. 128–130° (31 mm.), 87–90° (2.5 mm.), $n^{25}D$ 1.4776, was obtained in 48% yield from *p*-fluorotoluene.

Anal. Calcd. for $C_{10}H_{11}FO_2$: C, 65.92; H, 6.08. Found: C, 66.11; H, 6.53.

Ethyl 4-Amino-3-nitrophenylacetate.—A suspension of 64 g. of 4-amino-3-nitrophenylacetic acid[34] in 1 l. of absolute ethanol was saturated with dry hydrogen chloride. After the mixture had stood overnight, it was chilled and the white crystalline product collected. This material was stirred with a mixture of 500 ml. of ether and excess sodium bicarbonate solution. The yellow ether solution was separated, dried and evaporated, leaving 50 g. (69%) of bright yellow ester; m. p. 80–81°

(32) Fieser and Bowen, THIS JOURNAL, **62**, 2106 (1940).
(33) Asinger, *J. prakt. Chem.*, **142**, 297 (1935).
(34) Gabriel, *Ber.*, **15**, 839 (1882).

Anal. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.40. Found: C, 53.30; H, 5.04.

4-Methoxy-3-nitrophenylacetic Acid.—This acid was prepared by converting 4-methoxy-3-nitrobenzyl chloride[35] to the nitrile followed by hydrolysis with hydrochloric acid. It was recrystallized from methyl acetate–petroleum ether; m. p. 122–125°.

Anal. Calcd. for $C_9H_9NO_5$: C, 51.18; H, 4.29; N, 6.63. Found: C, 51.21; H, 4.34; N, 6.85.

Methyl *p*-Methylmercaptophenylacetate.—Twenty-four and eight-tenths grams of thioanisole,[36] 150 ml. of carbon disulfide and 24 g. of acetyl chloride were mixed and stirred in an ice-bath. Then 30 g. of anhydrous aluminum chloride was added portionwise. After stirring four hours, the reaction mixture was decomposed with ice and hydrochloric acid, the carbon disulfide removed by distillation and the solid residue was extracted with ether. Evaporation of the ether gave *p*-methylmercaptoacetophenone, which was recrystallized from ether–petroleum ether; m. p. 72–75°.

Anal. Calcd. for $C_9H_{10}OS$: C, 65.02; H, 6.06. Found: C, 65.06; H, 5.96.

This ketone was converted to the acid by means of the modified Willgerodt[25] method. A mixture of 49.8 g. of *p*-methylmercaptoacetophenone, 9.6 g. of sulfur and 27 ml. of morpholine was heated under reflux overnight. Then 400 ml. of concentrated hydrochloric acid and 300 ml. of water were added and the mixture was again heated under reflux overnight. The resulting oil was extracted with ether and the ether extracts were treated with an excess of dilute sodium hydroxide solution. Acidification of this alkaline solution yielded 25 g. of *p*-methylmercaptophenylacetic acid which melted at 92–94° after recrystallization from methyl acetate–petroleum ether.

Anal. Calcd. for $C_9H_{10}O_2S$: C, 59.31; H, 5.53. Found: C, 59.71; H, 5.25.

Esterification with methanol and dry hydrogen chloride gave methyl *p*-methylmercaptophenylacetate, b. p. 179–181° (3 mm.).

Anal. Calcd. for $C_{10}H_{12}O_2S$: C, 61.19; H, 6.16. Found: C, 60.76; H, 6.13.

***m*-Trifluoromethylphenylacetic Acid.**—A solution of methylmagnesium iodide was prepared from 60 g. of methyl iodide and 9 g. of magnesium in 400 ml. of ether. A solution of 51.5 g. of *m*-trifluoromethylbenzonitrile[37] in 50 ml. of ether was added with stirring over a period of one hour to the Grignard reagent. After three hours the reaction mixture was poured into a mixture of 500 g. of ice and 100 ml. of concentrated hydrochloric acid. The ether layer was separated, washed with water, dried and distilled. The fraction boiling at 190–210° was collected. This liquid was redistilled and the *m*-trifluoromethylacetophenone collected at 198–202°; yield, 28 g. (50%).

Anal. Calcd. for $C_9H_7F_3O$: C, 57.45; H, 3.75. Found: C, 57.20; H, 3.82.

A mixture of 90 g. (0.5 mole) of *m*-trifluoromethylbenzoic[37] acid (obtained in 78% yield by carbonation of *m*-trifluoromethylphenylmagnesium bromide[38]) and 80 g. of thionyl chloride was allowed to stand overnight. After removal of the excess thionyl chloride the *m*-trifluoromethylbenzoyl chloride distilled at 184–186° (750 mm.). The yield was 93.5 g. (95.5%). This product, dissolved in 100 ml. of dry ether, was added dropwise with stirring to dimethylcadmium prepared from 25 g. of magnesium, 100 g. of methyl bromide and 110 g. (0.6 mole) of cadmium chloride in a total of 700 ml. of ether. The reaction mixture was stirred for one-half hour, decomposed with ice and hydrochloric acid and worked up to yield 76 g. (91%) of *m*-trifluoromethylacetophenone.

(35) Quelet and Germain, *Compt. rend.*, **202**, 1442 (1936); Darzens, *ibid.*, **208**, 818 (1939).

(36) Brand and Vogt, *J. prakt. Chem.*, [2] **107**, 387 (1924).

(37) Swarts, *Bull. classe sci. acad. roy. Belg.*, [3] **35**, 375 (1898); [*Chem. Centr.*, **69**, II, 26 (1898)].

(38) Simons and Ramler, THIS JOURNAL, **65**, 389 (1943).

A mixture of 10 g. of *m*-trifluoromethylacetophenone, 2 g. of sulfur and 5.3 g. of morpholine was heated at 135° for sixteen hours. Then 30 ml. of glacial acetic acid and 50 ml. of concentrated hydrochloric acid were added and the mixture was boiled under reflux for about seven hours. After partial evaporation under vacuum, the mixture was diluted with 500 ml. of water and extracted with ether. The ether extract was washed with water and extracted with a solution of 10 g. of sodium carbonate in 150 ml. of water. Acidification of the carbonate extract gave an oil which crystallized after standing. The yield was 9.6 g. (89%). Recrystallization from petroleum ether gave the acid, m. p. 72–73°.

Anal. Calcd. for $C_9H_7F_3O_2$: C, 52.95; H, 3.45. Found: C, 53.10; H, 3.38.

Ethyl *p*-Phenoxyphenylacetate.—A mixture of 60 g. of *p*-phenoxyacetophenone, b. p. 160–161° (0.3 mm.),[39] 13 g. of sulfur and 10 ml. of morpholine was heated under reflux overnight. The resultant crude product was hydrolyzed by heating under reflux for two days with 75 g. of potassium hydroxide dissolved in 75 ml. of water and 600 ml. of ethanol. The alcohol was removed by distillation and the residue was treated with water. Acidification of the aqueous phase gave the acid which was esterified with ethanol and sulfuric acid; yield, 25 g.; b. p. 173–174° (0.2 mm.).

Anal. Calcd. for $C_{16}H_{16}O_3$: C, 74.97; H, 6.29. Found: C, 75.30; H, 6.45.

Methyl *p*-Anisoylaminophenylacetate.—*p*-Aminophenylacetic acid was acylated with anisoyl chloride by the Schotten–Baumann method. A suspension of 23 g. of *p*-anisoylaminophenylacetic acid (m. p. 211–212°) in 100 ml. of methanol was treated with excess ethereal diazomethane and the methyl ester was obtained in quantitative yield as long colorless needles; m. p. 162°.

Anal. Calcd. for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72. Found: C, 68.34; H, 5.93.

Ethyl *p*-Phenylmercaptophenylacetate.—*p*-Phenylmercaptoacetophenone, b. p. 180° (1 mm.), was obtained by the Friedel–Crafts method from diphenyl sulfide and acetyl chloride as described above for *p*-methylmercaptoacetophenone.

Anal. Calcd. for $C_{14}H_{12}OS$: C, 73.64; H, 5.30. Found: C, 73.54; H, 4.93.

This ketone was converted to *p*-phenylmercaptophenylacetic acid by the usual procedure.[25] The acid was esterified with ethanol and sulfuric acid, and the ester distilled at 163° (0.65 mm.).

Anal. Calcd. for $C_{16}H_{16}OS$: C, 70.55; H, 5.92. Found: C, 70.04; H, 5.83.

Ethyl *p*-(γ-Diethylaminopropoxy)-phenylacetate.—Thirty-six grams of ethyl *p*-hydroxyphenylacetate was added to a solution of 11 g. of sodium methoxide in 300 ml. of absolute ethanol. Then 30 g. of γ-diethylaminopropyl chloride was added and the mixture was stirred and boiled under reflux overnight. Most of the alcohol was removed by distillation from a steam-bath and the residue was treated with water and ether. The ether layer was extracted with cold dilute hydrochloric acid and this acid extract was made alkaline with sodium hydroxide solution. The basic ester which separated was extracted with ether, dried and distilled *in vacuo;* b. p. 145–147° (0.3 mm.); yield, 24 g. The hydrochloride was made with anhydrous hydrogen chloride and recrystallized from ethanol–ether; m. p. 121°.

Anal. Calcd. for $C_{17}H_{28}ClNO_3$: N, 4.25. Found: N, 4.59.

N-(2′-Hydroxyethyl)-4-hydroxy-3-phenylazophenylacetamide.—A solution was prepared from 49 g. (0.25 mole) of N-(2-hydroxyethyl)-*p*-hydroxyphenylacetamide and 165 ml. of cold 10% sodium hydroxide solution. Simultaneously, a benzenediazonium chloride solution was made from 23 ml. of aniline, 270 g. of ice and 74 ml. of

(39) Kipper, *Ber.*, **38**, 2491 (1905).

concentrated hydrochloric acid by the addition of 18 g. of sodium nitrite in 36 ml. of water. Then 180 g. of cracked ice was added to the solution of the ethanolamide and the diazonium salt solution was added below the surface of the mixture. Stirring and cooling by the addition of more ice was maintained throughout the procedure. After the dark red mixture had stirred for three hours at 6–10°, the precipitate was collected and washed with water; yield 56.5 g.; m. p. 167–172° (micro block). After being recrystallized from benzene–ethanol, the melting point was 180–181.5°

N-(2'-Hydroxyethyl)-3,5-diacetomercuri-4-hydroxyphenylacetamide.—A solution was prepared from 49 g. (0.25 mole) of N-(2'-hydroxyethyl)-4-hydroxyphenylacetamide, 800 ml. of 50% ethanol, 40 ml. of acetic acid and 79.7 g. (0.25 mole) of mercuric acetate. After the mixture had stood at room temperature for twelve days, it was chilled and the precipitate was collected on a filter. This solid was then heated with 750 ml. of 50% alcohol containing 5% of acetic acid. The resulting solution was cooled, whereupon 51.4 g. of amide separated. In the melting point bath the product turned brown at 200° but remained unmelted at 280°. When the bath was heated rapidly, the compound partially melted at 240°.

Ethyl *p*-*t*-Butylphenylacetate.—A mixture of 87 g. of *p*-*t*-butylacetophenone,[40] 24 g. of sulfur and 68 ml. of morpholine yielded crude *p*-*t*-butylphenylacetic acid in the same manner as *m*-trifluoromethylphenylacetic acid was obtained. It was esterified with ethanol and sulfuric acid. The resulting ester distilled at 95° (0.47 mm.), and the yield was 19.4 g.

Anal. Calcd. for $C_{14}H_{20}O_2$: C, 76.32; H, 9.15. Found: C, 76.40; H, 9.27.

Ethyl *p*-*t*-Amylphenylacetate.—A mixture of 68.5 g. of *p*-*t*-amylacetophenone,[41] 19 g. of sulfur and 50 ml. of morpholine gave the acid which was converted to the ethyl ester exactly as was done in the case for ethyl *p*-*t*-butylphenylacetate; b. p. 124° (2 mm.); yield, 15.4 g.

Anal. Calcd. for $C_{15}H_{22}O_2$: C, 76.87; H, 9.46. Found: C, 76.64; H, 9.63.

Ethyl *p*-Allyloxyphenylacetate.—The acid was obtained from 90 g. of ethyl *p*-hydroxyphenylacetate and 70 g. of allyl bromide as in the case of *p*-benzyloxyphenylacetic acid. After saponification the free *p*-allyloxyphenylacetic acid was then isolated, dried and esterified by refluxing overnight with 500 ml. of absolute ethanol and 10 g. of benzenesulfonic acid. The resulting ethyl *p*-allyloxyphenylacetate boiled at 126–127° (0.5 mm.). The yield was 18.4 g.

Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.88; H, 7.32. Found: C, 70.54; H, 7.72.

Ethyl *p*-(β,γ-Dihydroxypropoxy)-phenylacetate.—A solution of 44 g. of ethyl *p*-allyloxyphenylacetate in 100 ml. of 70% acetone was treated at 20° with a solution of 22 g. of potassium permanganate in 300 ml. of 70% acetone. After the addition had been completed, acetic acid (8 g.) was added. The solution was filtered and the filtrate evaporated under reduced pressure. Water was added to the residue and the product was extracted with ether. After drying over magnesium sulfate, the solution was distilled to give the ester; b. p. 200° (0.2 mm.); yield 24.8 g.

Anal. Calcd. for $C_{13}H_{18}O_5$: C, 61.40; H, 7.13. Found: C, 61.85; H, 6.84.

Methyl Dithiophenylacetate.[42]—A Grignard solution was prepared from 3.5 moles of benzyl chloride in 1500 ml. of dry ether. It was cooled in an ice–salt-bath and treated with 268 g. (3.5 moles) of carbon disulfide over a period of forty-five minutes. The mixture was allowed to stand overnight in an ice-bath and then decomposed by the addition of 615 ml. of 6 *N* hydrochloric acid with cooling over a period of one hour. The ether layer was separated, filtered and washed twice with water. This ether solution was extracted with 1 l. of a cold 2.17 molar solution of sodium hydroxide. The aqueous extract, which was somewhat basic, was cooled in an ice-bath and stirred well while 200 g. (1.6 mole) of dimethyl sulfate was added over a period of fifteen minutes. The temperature was kept below 30°. The resulting mixture was extracted with ether and the organic solution was dried over magnesium sulfate and distilled. The yellow liquid boiled at 122–125° (3 mm.); yield 150 g. (23% of theory on the basis of the benzyl chloride used).

N-Methyl-N-*n*-propylthiophenylacetamide.—When 18.2 g. (0.1 mole) of methyl dithiophenylacetate was added to 15 g. of methyl-*n*-propylamine, the solution became hot and gas was evolved. After the initial reaction had subsided, the solution was heated to boiling. The reaction mixture was distilled *in vacuo*. The amide boiled at 155–158° (1.5 mm.); $n^{23.5}D$ 1.5876. The yield was 17.9 g. (86%).

Anal. Calcd. for $C_{12}H_{17}NS$: N, 6.76. Found: N, 6.31. Stimulation results, 0.8; differential assay[4] 1.03.

Thiophenylacetylation of Amino Acids.—The amino acid was weighed into a flask and exactly neutralized with standard 4 *N* sodium hydroxide solution. The solution was diluted with an equal volume of ethanol and a 10% molar excess of methyl dithiophenylacetate was added. The mixture was shaken for a period of a few minutes to several hours. It was evaporated under reduced pressure to half the original volume and then washed with ether. The aqueous solution was acidified with hydrochloric acid and the product was extracted with ether. After the ether solution had been dried over magnesium sulfate, it was evaporated to a small volume and diluted with petroleum ether to cause crystallization of the thiophenylacetylamino acid.

Thiophenylacetyl-D-penicillamine, yield 55%, m. p. 132–133°. *Anal.* Calcd. for $C_{13}H_{17}NO_2S$: N, 4.95. Found: N, 4.99.

Thiophenylacetyl-L-penicillamine, yield 61%, m. p. 133–134°.

Thiophenylacetyl-β,β-diethoxyalanine, yield 84%, m. p. 67.5–68°. *Anal.* Calcd. for $C_{16}H_{21}NO_4S \cdot 0.5H_2O$; C, 56.25; H, 6.87. Found: C, 56.47; H, 7.19.

Thiophenylacetyl-DL-valine, yield 95%, m. p. 102–103°. *Anal.* Calcd. for $C_{13}H_{17}NO_2S$: N, 5.58. Found: N, 5.42.

Thiophenylacetyl-DL-isoleucine, yield 75%, m. p. 95–96°. *Anal.* Calcd. for $C_{14}H_{19}NO_2S$: N, 5.28. Found: N, 5.27.

Stimulation results have been reported[43] for these derivatives.

Formation of *p*-Hydroxybenzylpenicillin in Presence of N-(2-Hydroxyethyl)-*p*-hydroxyphenylacetamide.—Ten 250-ml. erlenmeyer flasks each containing 70 ml. of medium (25 g. of lactose, 20 g. of corn steep solids, 2 g. of calcium carbonate, 0.044 g. of zinc sulfate heptahydrate and 0.25 g. of N-(2-hydroxyethyl)-*p*-hydroxyphenylacetamide per liter) were inoculated with 10 ml. of a two-day vegetative growth of *P. notatum* NRRL 1976. After four days of incubation with shaking at 25° the broth was collected and was found to assay 73 u./ml.

The chilled broth, 780 ml., was extracted with an equal volume of amyl acetate at *p*H 2.2. The amyl acetate was extracted with two 185-ml. portions of *p*H 7.1 phosphate buffer. At this point 88% of the original penicillin was contained in the aqueous buffer solution.

The chilled buffer solution was acidified and extracted with two 185-ml. portions of chloroform followed by 370 ml. of amyl acetate. The sodium salt was prepared from the amyl acetate solution yielding 3500 units or 8% of the original activity. The differential assay value of this fraction was 1.39. An authentic sample of *p*-hydroxybenzylpenicillin gave average differential assay values of 1.4.

(40) Verley, *Bull. soc. chim. France*, [3] **19**, 73 (1898).

(41) Weygand, Mensdorf and Strobelt, *Ber.*, **68**, 1825 (1935).

(42) Houben and Schultze, *Ber.*, **43**, 2484 (1910).

(43) "The Chemistry of Penicillin," University of Princeton Press, Princeton, N. J., 1948, Chapter 19.

Control experiments in which no precursor was used gave no appreciable quantities of *p*-hydroxybenzylpenicillin.

Acknowledgment.—The authors are grateful for the valuable assistance given by Charlotte M. Harris, Dorothea S. Huff, Marjorie J. Mann and John O'Brien. The microanalyses were performed by W. L. Brown and H. L. Hunter. We also thank Dr. G. H. A. Clowes and Dr. E. C. Kleiderer for their interest and helpful suggestions.

Summary

The N-2-hydroxyethylamide and/or valine derivatives of a series of substituted phenylacetic acids have been prepared and tested as penicillin precursors. The effect of these materials on the formation of new penicillins has been indicated and discussed.

It has been demonstrated that N-2-hydroxyethyl-*p*-hydroxyphenylacetamide serves as a precursor for penicillin X.

INDIANAPOLIS, INDIANA RECEIVED MAY 3, 1948

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Biosynthesis of Penicillins. VI. N-2-Hydroxyethylamides of Some Polycyclic and Heterocyclic Acetic Acids as Precursors[1]

BY REUBEN G. JONES, QUENTIN F. SOPER, OTTO K. BEHRENS AND JOSEPH W. CORSE[2]

In connection with the biosynthetic preparation of new penicillins, a wide variety of acids and their derivatives were tested as possible precursor substances. This paper describes the N-2-hydroxyethylamides of a number of polycyclic and heterocyclic analogs of phenylacetic acid. In addition several new polycyclic and heterocyclic acetic acids are reported.

Of a large number of derivatives of phenylacetic acid, the N-2-hydroxyethylamide was among the most effectively utilized by *P. notatum* NRRL 1976 for the production of benzylpenicillin.[3] On this basis the N-2-hydroxyethylamides of the present series of acids were chosen as appropriate derivatives for testing. The compounds of Table I were tested in the manner previously described[3,4] using *P. notatum* NRRL 1976 and *P. chrysogenum* Q176. Only a few of these compounds appeared to be utilized readily by the mold for the formation of new penicillins. 2-Thiopheneacetic acid and 2-thiopheneacetyl-DL-valine as well as N-(2'-hydroxyethyl)-2-thiopheneacetamide were converted by the mold to 2-thiophenemethylpenicillin, the isolation of which has been described.[5]

Several of the compounds appeared to effect some increase in penicillin yield (last column, Table I) or to change the differential assay value[3] of the crude penicillin produced in their presence. In some of these cases the crude penicillins were subjected to separation in the "Craig apparatus."[4] This analysis failed to show that significant quantities of new penicillins were formed from 2-furanacetic acid, 6-quinolineacetic acid, 1-bromo-2-naphthaleneacetic acid or N-(2'-hydroxyethyl)-1-pyrroleacetamide using *P. chrysogenum* Q176, or from N-(2'-hydroxyethyl)-2-furanacetamide using *P. notatum* NRRL 1976. On the other hand, when NRRL 1976 was grown in the presence of N-(2'-hydroxyethyl)-2-naphthaleneacetamide a new penicillin fraction was found by the Craig technique. This new penicillin has not as yet been isolated in a pure condition. Its differential assay value appears to be about 0.6.

In a number of cases efforts were made to isolate new penicillins by separations on silica gel columns.[3] From this work it has been concluded that, under the conditions of testing which were used, no significant quantities of new penicillins were formed from the N-2-hydroxyethylamides of 3-pyridineacetic acid, 5-benzimidazoleacetic acid, 7-hydroxy-4-coumarinacetic acid or 2-furanacetic acid.

In addition to the compounds listed in Table I, 3-coumarinacetic acid,[6] tryptamine and histamine were tested as possible precursor substances, In each case there was no stimulation in penicillin yield.

Polycyclic Acetic Acids.—The new acids in this group include five substituted 2-naphthaleneacetic acids and two phenanthreneacetic acids. 6-Methoxy-2-naphthaleneacetic, 5,6,7,8-tetrahydro-2-naphthaleneacetic, 2-phenanthreneacetic and 3-phenanthreneacetic acids were obtained in satisfactory yields from the corresponding methyl ketones by the modified Willgerodt method.[6a] 6-Amino-2-methylnaphthalene served as a starting point in the synthesis of 6-fluoro- and 6-bromo-2-naphthylacetic acids. The amino group was replaced with the halogens through diazonium reactions: the 6-halo-2-methylnaphthalenes were monobrominated in the side chain, and

(1) For the preceding paper of this series, see: Corse, Jones, Soper, Whitehead and Behrens, THIS JOURNAL, **70**, 2837 (1948).

(2) Present address: University of California at Los Angeles, Los Angeles, California.

(3) Behrens, Corse, Jones, Mann, Soper, Van Abeele and Chiang, *J. Biol. Chem.*, **175**, 751 (1948).

(4) Behrens, Corse, Huff, Jones, Soper and Whitehead, *ibid.*, **175**, 771 (1948).

(5) Behrens, Corse, Edwards, Garrison, Jones, Soper, Van Abeele and Whitehead, *ibid.*, **175**, 793 (1948).

(6) Dey and Sankaranarayanan, *J. Indian Chem. Soc.*, **8**, 817 (1931); [*C. A.*, **26**, 3499 (1932)].

(6a) Schwenk and Bloch, THIS JOURNAL, **64**, 3051 (1942).