image of a flexible active center conforms with the induced fit hypothesis of Koshland.[38] We are suggesting that the approach of the substrate toward the serine hydroxyl triggers the shift in configuration.

(38) *Ibid.*, p. 334.

We wish to acknowledge the technical assistance of Mr. Marvin Lache and the able assistance of Dr. Frances Edel and Mr. Nicholas Kokowsky in the preparation of a number of the compounds reported in this paper.

[CONTRIBUTION FROM THE RESEARCH DIVISION, BRISTOL LABORATORIES, DIVISION OF BRISTOL-MYERS COMPANY]

Derivatives of 6-Aminopenicillanic Acid. I. Partially Synthetic Penicillins Prepared from α-Aryloxyalkanoic Acids

BY Y. G. PERRON, W. F. MINOR, C. T. HOLDREGE, W. J. GOTTSTEIN, J. C. GODFREY, L. B. CRAST, R. B. BABEL AND L. C. CHENEY

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The preparation of a number of new racemic α-aryloxyalkanoic acids (I) is reported. These and some previously described racemic α-aryloxyalkanoic acids were used for the N-acylation of 6-aminopenicillanic acid (6-APA) (II). The new diastereoisomeric α-aryloxyalkylpenicillins thus produced (III) were isolated as potassium salts. The two possible diastereoisomers of one of these new penicillins, namely, α-phenoxyethylpenicillin, were prepared in pure form and their distinctive physical properties are recorded.

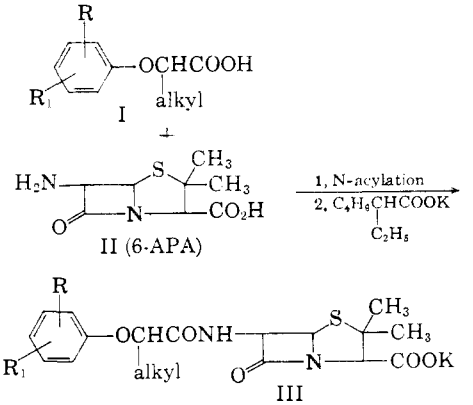
It has been known for some time that new penicillins could be produced by biosynthetic methods, that is by feeding the mold a variety of suitable precursors which could be incorporated into the penicillin molecule. Although numerous penicillins have been prepared in this manner, the method was found to be limited by the structural type of precursors that could be utilized by the mold.[1,2]

An important contribution to the problem of penicillin preparations was achieved recently when Sheehan and Henery-Logan reported the total synthesis of penicillin V.[3] This synthesis has opened a way by which an unlimited number of novel penicillins or analogs may now be prepared. However, at this time the procedure does not readily lend itself to the large scale preparation of penicillins of potential therapeutic value.

The recent disclosure by Batchelor, *et al.*,[4] that 6-aminopenicillanic acid (6-APA) (II) could be obtained by fermentation made available a most useful chemical intermediate which can be used for the practical preparation of a number of new penicillins not amenable to production by biosynthetic methods.

We have undertaken an extensive synthetic program using 6-APA as starting material, hoping to obtain novel penicillins that have one or more of the following advantages: (1) broader antimicrobial spectra, (2) more favorable absorption patterns and (3) reduced undesirable side effects. We wish to report a series of new penicillins prepared by the N-acylation of 6-APA (II) with a variety of α-aryloxyalkanoic acids (I). The intermediate α-aryloxyalkanoic acids used are described in Table I. These acids have been prepared by four different methods which are depicted in Table I as A, B, C and D. It should be noted that method C was found to be the most general and most reliable method especially when steric hindrance was present.

The new penicillins (III) described in Table II have been prepared by condensation of the α-aryloxyalkanoic acids with 6-APA through the acid chloride (method A) or the mixed carboxylic–carbonic anhydride[5] (method B) using either ethyl or isobutyl chloroformate. In all cases the reaction products were isolated as potassium salts by cation interchange with potassium 2-ethylhexanoate.



The infrared spectra (KBr) of the new potassium α-aryloxyalkylpenicillins of Table II showed a strong adsorption at 5.55–5.68 μ which is characteristic of the β-lactam ring. The presence of this four-membered lactam ring was further demonstrated by the quantitative hydroxylamine assay for penicillins.[6]

(1) H. T. Clarke, J. R. Johnson and R. Robinson, Editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 657.

(2) J. E. Philip, *et al.*, *J. Biol. Chem.*, **189**, 479 (1951); O. K. Behrens and M. J. Kingkade, *ibid.*, **176**, 1047 (1948). This last paper provides leading references to preceding papers in this series.

(3) J. C. Sheehan and K. R. Henery-Logan, THIS JOURNAL, **79**, 1262 (1957); **81**, 3089 (1959).

(4) F. R. Batchelor, F. P. Doyle, J. H. C. Nayler and G. N. Rolinson, *Nature*, **183**, 257 (1959).

(5) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951); J. R. Vaughan, Jr., *et al.*, THIS JOURNAL, **73**, 3547, 5553 (1951); **74**, 676 (1952); V. du Vigneaud, *et al.*, *ibid.*, **75**, 4879 (1953); **76**, 3115 (1954); D. S. Tarbell, *et al.*, *J. Org. Chem.*, **23**, 1149, 1152 (1958); **24**, 774 (1959). The last three papers contain interesting studies of this reaction.

(6) J. H. Ford, *Anal. Chem.*, **19**, 1004 (1947).

Although we have prepared numerous new penicillins from α-aryloxyalkanoic acids and 6-APA for biological investigation, the ones reported herein are representative of those which have been obtained in analytically pure form.

Since the racemic α-aryloxyalkanoic acids were used in these preparations, the new penicillins obtained were diastereoisomeric mixtures. It was of interest to prepare both epimeric forms of the reaction product between α-phenoxypropionic acid and 6-APA, namely, potassium α-phenoxyethylpenicillin,[7,8] since this material was shown to be of therapeutic value.[9] Thus, the racemic α-phenoxypropionic acid was resolved and the dextrorotatory acid was obtained by a method previously described by Fourneau and Sandulesco,[10] while the levorotatory acid was isolated by the procedure of Fredga and Matell.[11] These latter authors stated that dextro-α-phenoxypropionic acid is probably related to D-(−)-lactic acid although no rigid experimental proof is provided for this assignment. It should then be noted that the notations D and L, referring to the configurations of the side-chains of the two epimeric potassium α-phenoxyethylpenicillins, are provisional and are used here only to distinguish between the two diastereoisomers.

The optically pure enantiomeric α-phenoxypropionic acids then were condensed in turn with 6-APA. The method used for this coupling was essentially that of Vaughan[12] which was developed for the preparation of optically active peptides. This method consists of treating the mixed carboxylic–carbonic anhydride obtained from the optically active acid and isobutyl chloroformate with 6-APA. Although the reactions were carried out under a variety of experimental conditions, the formation of a small amount of racemized material could not be prevented. The occurrence of some racemization also was observed by Vaughan[13] during the synthesis of optically active peptides. However, the two optically pure potassium α-phenoxyethyl penicillins could be obtained by successive recrystallizations of the reaction products from acetone–water and butanol–water mixtures; the progress of the purification was followed by infrared, optical rotation and phase-solubility determinations.[14]

The two epimeric potassium α-phenoxyethylpenicillins possess different physical properties as well as distinctive antimicrobial activities.[15] The infrared absorption spectra of the two crystalline diastereoisomers show an analogous absorption in the functional group region of the spectrum while the location and intensity of the bands in the "fingerprint" region are quite distinctive.

Acknowledgments.—We wish to express our appreciation to F. M. Palermiti and D. L. Evans for infrared spectra, to R. M. Downing for the elemental analyses, to H. A. Frediani for the phase-solubility determinations, and to C. Rowley and P. Lau for valuable technical assistance.

Experimental[16]

α-Aryloxyalkanoic Acids.—Four general methods were used for the synthesis of the α-aryloxyalkanoic acids of Table I. Methods A and B consisted in the reaction of a sodium phenolate with the sodium salt of an α-haloalkanoic acid, while methods C and D required the reaction of a sodium phenolate with an ethyl α-haloalkanoate, with subsequent hydrolysis of the ester formed. α-Bromoalkanoic acids were used in A and B whenever they were available since the corresponding α-chloro acids gave slightly lower yields under the same conditions. No attempt was made to determine the conditions necessary for optimum yields.

The majority of the acids prepared were crystalline solids which could be purified by recrystallization from benzene–petroleum ether, or from isopropyl alcohol–water. Those compounds which did not crystallize were distilled *in vacuo*. For each of the four methods used there is given a representative preparation.

(A) **α-(4-Benzyloxyphenoxy)-propionic Acid.**—To a stirred solution of 140 g. (0.7 mole) of hydroquinone monobenzyl ether and 76 g. (0.7 mole) of α-chloropropionic acid in 400 ml. of ethanol there was added rapidly a chilled solution of of 80 g. (2.0 moles) of sodium hydroxide in 150 ml. of water. The mixture was refluxed for 20 hr. and the alcohol was removed under reduced pressure. The residue was dissolved in water, acidified to *p*H 2 with concentrated hydrochloric acid and the precipitated solid was extracted into several portions of ether. The combined ethereal extracts were extracted thrice with 5% sodium bicarbonate, ether was removed from the combined extracts *in vacuo* and the bicarbonate solution was acidified to *p*H 2 with concentrated hydrochloric acid, precipitating a crystalline solid. The dried, crude product was recrystallized from benzene–petroleum ether (b.p. 60–71°); yield 140 g. (74%), m.p. 134–135°. See Table I for the analysis.

(B) **α-(3-Trifluoromethylphenoxy)-propionic Acid.**—A solution of 20 g. (0.5 mole) of sodium hydroxide in 125 ml. of water was added to 76.5 g. (0.5 mole) of α-bromopropionic acid cooled in an ice-bath, the temperature being kept below 30°. To the resultant solution there was added 81 g. (0.5 mole) of 3-trifluoromethylphenol in 100 ml. of 5 *M* sodium hydroxide. The mixture was heated for 20 hr. on the steam-bath, cooled, acidified to *p*H 2 with concentrated hydrochloric acid and extracted several times with ether. The combined ethereal solutions were extracted three times with 5% aqueous sodium bicarbonate. The combined extracts were acidified to *p*H 2 with concentrated hydrochloric acid after removal of dissolved ether *in vacuo*. The oily precipitate did not crystallize and was purified by ether extraction, drying over anhydrous sodium sulfate and distillation *in vacuo*. There was obtained 182.5 g. (78%) of viscous yellow oil of b.p. 140–144° (8 mm.). This oil crystallized to a yellowish solid of m.p. 63–66°, which was not purified further. The analysis is reported in Table I.

(C) **α-Phenoxy-*n*-valeric Acid.**—A stirred mixture of 44.2 g. (0.47 mole) of phenol and 24 g. (0.60 mole) of flake sodium hydroxide in 600 ml. of toluene was refluxed under a Dean–Stark trap until the theoretical amount of water (8.5 ml.) had been collected. With moderation of heating there was then added 98 g. (0.47 mole) of ethyl α-bromo-*n*-valerate at a rate which maintained moderate reflux. The mixture was refluxed for 16 hr., cooled to 60° and there was added consecutively 100 ml. of methanol and a solution of 29 g. (0.72 mole) of sodium hydroxide in 120 ml. of water. The resulting solution was refluxed vigorously for 2 hr. to complete the saponification and diluted with 500

(7) According to the suggested penicillanic acid nomenclature [J. C. Sheehan, K. R. Henery-Logan and D. A. Johnson, THIS JOURNAL, **75**, 3292 (1953)] the systematic name for this compound is potassium 6-(α-phenoxypropionamido)-penicillanate.

(8) The trade name of Bristol Laboratories, for potassium α-phenoxyethylpenicillin is Syncillin.

(9) (a) M. H. Pindell, D. E. Tisch and J. B. Hoekstra, "Antibiotics, Annual 1959–1960," Medical Encyclopedia, New York, p. 119; (b) E. M. Morigi, W. B. Wheatley and H. Albright, *ibid.*, 1959–1960, p. 127; (c) G. A. Cronk, D. E. Naumann, H. Albright and W. B. Wheatley, *ibid.*, 1959–1960, p. 133.

(10) E. Fourneau and G. Sandulesco, *Bull. soc. chim. France, Ser. 4*, **31**, 988 (1922).

(11) A. Fredga and M. Matell, *Arkiv. Kemi*, **4**, 325 (1952).

(12) J. R. Vaughan, Jr., THIS JOURNAL, **74**, 6137 (1952).

(13) J. R. Vaughan, Jr., and J. A. Eichler, *ibid.*, **75**, 5556 (1953).

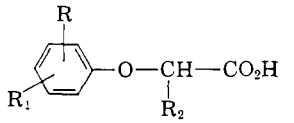
(14) H. A. Frediani, *Ann. chim.* (*Rome*), **42**, 692 (1952).

(15) A. Gourevitch, G. A. Hunt and J. Lein, "Antibiotics Annual 1959–1960," Medical Encyclopedia, New York, N. Y., p. 111.

(16) Melting points are uncorrected.

TABLE I

α-ARYLOXYALKANOIC ACIDS



R	R_1	R_2	Method	Yield, %	M.p.[a] or b.p., °C. (mm.)	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
H	H	CH_3	A[b]	86	116–117					
H	H	C_2H_5	A[c]	69	82–83					
H	H	n-C_3H_7	C[d]	85	114.5–116					
H	H	n-C_4H_9	D[e]	11	74.5–75.5					
2-Cl	H	CH_3	A[f]	28	114.5–116					
4-Cl	H	CH_3	A[g]	54	115–116					
3-CF_3	H	CH_3	B	78	140–144(8)	$C_{10}H_9F_3O_3$	51.2	51.1	3.89	4.17
4-CH_3O	H	CH_3	B[h]	63	92–93					
4-$C_6H_5CH_2O$	H	CH_3	A	74	134–135	$C_{16}H_{16}O_4$	70.6	71.0	5.92	6.09
4-C_6H_{11}	H	CH_3	A	63	125–126	$C_{15}H_{20}O_3$	72.6	72.0	8.12	8.44
4-CH_3CONH	H	CH_3	B	67	176–177	$C_{11}H_{13}NO_4$	59.2	59.3	5.86	5.93
4-NO_2	H	CH_3	B[i]	21	140–141					
4-NO_2	3-CF_3	CH_3	A	60	90–91	$C_{10}H_8F_3NO_5$	43.0	43.6	2.89	3.12
3-CH_3	5-CH_3	CH_3	B[j]	41	118–119					
4-C_6H_5	2-Cl	CH_3	C	61	145–146	$C_{15}H_{13}ClO_3$	65.1	65.4	4.73	4.76
2-$C_6H_5CH_2$	4-Cl	CH_3	C	46	115–116	$C_{16}H_{15}ClO_3$	66.0	66.3	5.37	5.37
4-CH_3O	2-C_6H_5CO	CH_3	C	31	130–131	$C_{17}H_{16}O_5$	68.0	68.2	5.37	5.35
4-Cl-3,5-xylyl		CH_3	B[k]	37	136–138					
Pentachloro		CH_3	C	60	173–174	$C_9H_5Cl_5O_3$	32.0	32.1	1.50	1.62

[a] All melting points and boiling points are uncorrected. [b] A. Fredga and M. Matell, *Arkiv. Kemi*, **4** (#20), 325 (1952); m.p. 116°. [c] C. H. Fawcett, *et al.*, *Ann. Appl. Biol.*, **40**, 231 (1953), reported m.p. 80°. [d] M. Matell, *Arkiv. Kemi*, **8**, 79 (1955), reported m.p. 113.5–114°. [e] M. Matell, *ibid.*, **6**, 375 (1953); m.p. 73–74°. [f] Reference *b* reported m.p. 113.5–114.5°. [g] Reference *b*, m.p. 114.5–115°; the same m.p. was reported by M. Matell, *Arkiv. Kemi*, **7**, 437 (1954). [h] H. Sobotka and J. Austin, THIS JOURNAL, **74**, 3813 (1952); m.p. 90°. [i] E. Fourneau and G. Sandulesco, *Bull. soc. chim.*, **33**, 459 (1923); m.p. 130–140°; reference *d* reported m.p. 142.5–143°. [j] P. W. Zimmerman, A. E. Hitchcock and E. K. Harvill, *Contrib. Boyce Thompson Inst.*, **13**, 273 (1944); m.p. 119–120°. [k] M. E. Synerholm and P. W. Zimmerman, *ibid.*, **14**, 91 (1945); m.p. 137–138°.

ml. of water. The alkaline aqueous layer was separated, cooled and acidified to *p*H 2 with 6 *N* sulfuric acid. The precipitated oil crystallized rapidly and was collected, washed with water and dried; yield 88 g. (96.5%). One recrystallization from benzene–petroleum ether (b.p. 60–71°) yielded 77 g. (85%) of product of m.p. 114–116° (lit.[17] 113.5–114°).

(D) α-Phenoxy-*n*-caproic Acid.—To a solution of sodium ethoxide prepared by dissolving 6.9 g. (0.3 mole) of sodium in 120 ml. of absolute ethanol, there was added consecutively 28.2 g. (0.3 mole) of phenol and 67 g. (0.3 mole) of ethyl α-bromo-*n*-caproate. The mixture was refluxed for 18 hr., 120 ml. of 2 *N* sodium hydroxide was added and reflux was continued for one hour. The ethanol was removed under reduced pressure and the aqueous residue was acidified to *p*H 2 with concentrated hydrochloric acid. The precipitated oil was purified as in A and B, and after being taken up in ether and dried over anhydrous sodium sulfate the product was obtained as a crystalline solid. After one recrystallization from petroleum ether (b.p. 60–71°) the material weighed 24.6 g. (60%), m.p. 74.5–75.5° (lit.[18] 73–74°).

Resolution of DL-α-Phenoxypropionic Acid. *d*-α-Phenoxypropionic Acid.—The method of Fourneau and Sandulesco[10] was adopted for the preparation of this optical isomer. From 226 g. of yohimbine hydrochloride there was obtained 12 g. of optically pure *d*-α-phenoxypropionic acid, m.p. 86.5–88°, $[\alpha]^{25}D$ +39.8° (*c* 1 in abs. alcohol) (lit.[10] $[\alpha]^{20}D$ +39.3°).

***l*-α-Phenoxypropionic acid** was isolated by a procedure essentially that of A. Fredga and Matell.[11] A product was obtained by evaporation under reduced pressure of the mother liquor from the isolation of the *d*-α-phenoxypropionic acid yohimbine salt. This material was treated with dilute sulfuric acid and extracted with ether to give an acid having $[\alpha]^{24}D$ −25.3° (*c* 1 in abs. alcohol). When 27 g. of this material was slurried with chloroform at room temperature and filtered there was obtained 10 g. of insoluble DL-α-phenoxypropionic acid of m.p. 117–119°, and 17 g. of soluble material by evaporation of the chloroform. The latter was slurried with 150 ml. of boiling cyclohexane and filtered, removing 1.4 g. of insoluble material. Evaporation of the filtrate left 14.7 g. of *l*-α-phenoxypropionic acid, m.p. 86–87.5°, $[\alpha]^{23.5}D$ −39.5° (*c* 1 in abs. alcohol) (lit.[11] $[\alpha]^{20}D$ −39.3°).

DL-α-Phenoxypropionyl Chloride.—In a 2-liter three-necked flask equipped with a stirrer, condenser ($CaCl_2$ tube) and a dropping funnel, 166 g. of DL-α-phenoxypropionic acid, 800 ml. of benzene and 2 ml. of pyridine were added. The mixture was stirred and heated to reflux, and 107.4 ml. of thionyl chloride was added dropwise during 30 minutes. When the addition was completed, refluxing was continued for 1 hour. The benzene was taken off under reduced pressure and the oily residue was distilled *in vacuo* yielding 146 g. of material, b.p. 114–115° at 18 mm.

Potassium α-Aryloxyalkylpenicillins.—The potassium α-aryloxyalkylpenicillins described in Table II were prepared by two general methods. Method A consisted in converting the α-aryloxyalkanoic acid into an acid chloride and treating it with 6-aminopenicillanic acid (6-APA) in aqueous acetone solution containing an excess of sodium bicarbonate. Method B was carried out by treatment of the aryloxyalkanoic acid with either ethyl or isobutyl chloroformate in the presence of triethylamine in a suitable solvent such as dimethylformamide, dioxane–acetone or tetrahydrofuran. The mixed carboxylic–carbonic anhydride thus produced then was caused to react with an aqueous solution of the triethylammonium salt of 6-APA. In both methods A and B the penicillin as the free acid was extracted into a water-immiscible organic solvent and then precipitated as the potassium salt by the addition of potassium 2-ethylhexanoate.

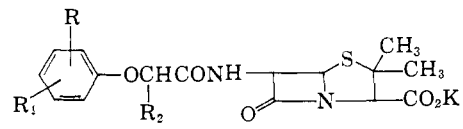
Method A was found to give higher yields and to be most general since method B gave less favorable results in those cases where the carboxyl group of the α-aryloxyalkanoic

(17) M. Matell, *Arkiv. Kemi*, **8**, 79 (1955).

(18) M. Matell, *ibid.*, **6**, 375 (1953).

TABLE II

POTASSIUM α-ARYLOXYALKYLPENICILLINS



R	R_1	R_2	Method	Yield, %	Decompn.,[a] °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
H	H	CH_3	A	76	230–232	$C_{17}H_{19}KN_2O_5S$[e]	50.74	50.81	4.72	4.88
H	H	C_2H_5	B	25	195–197	$C_{18}H_{21}KN_2O_5S$	51.85	51.73	5.10	5.61
H	H	n-C_3H_7	B	41	170–175	$C_{19}H_{23}KN_2O_5S\cdot H_2O$[d]	50.90	51.25	5.61	5.88
H	H	n-C_4H_9	B	50	>160	$C_{20}H_{25}KN_2O_5S$	54.03	53.95	5.67	5.64
2-Cl	H	CH_3	A	50	229–230	$C_{17}H_{18}ClKN_2OS$	46.75	46.99	4.15	4.44
4-Cl	H	CH_3	A	61	224–225	$C_{17}H_{18}ClKN_2O_5S$	46.75	46.85	4.15	4.28
2-Cl[b]	4-Cl	CH_3	B	61	203–205	$C_{17}H_{17}Cl_2KN_2O_5S\cdot H_2O$[d,f]	41.72	41.73	3.91	3.77
2-Cl[b]	5-Cl	CH_3	B	45	200–204	$C_{17}H_{17}Cl_2KN_2O_5S$	43.31	43.25	3.63	3.78
2-Br[b]	4-Br	CH_3	B	42	217–218	$C_{17}H_{17}Br_2KN_2O_5S$[g]	36.42	36.50	3.06	3.18
2,4,6-Trichloro[b]		CH_3	B	10	163–165	$C_{17}H_{16}Cl_3KN_2O_5S$	40.36	40.80	3.19	3.55
Pentachloro		CH_3	B	35	189–192	$C_{17}H_{14}Cl_5KN_2O_5S$	35.52	35.50	2.46	2.98
3-CF_3	H	CH_3	B	26	188–190	$C_{18}H_{18}F_3KN_2O_5S$	45.95	46.06	3.83	4.04
4-CH_3O	H	CH_3	B	34	211–214	$C_{18}H_{21}KN_2O_6S$	49.98	49.66	4.90	5.10
4-$C_6H_5CH_2O$	H	CH_3	A	79	228–229	$C_{24}H_{25}KN_2O_6S\cdot H_2O$[d]	54.75	54.98	5.17	5.02
4-t-C_4H_9[c]	H	CH_3	B	46	219–220	$C_{21}H_{27}KN_2O_5S$	55.02	54.52	5.89	5.98
4-t-C_5H_{11}[c]	H	C_2H_5	B	33	>125	$C_{23}H_{31}KN_2O_5S$	56.79	56.28	6.37	6.55
4-Cyclohexyl	H	CH_3	A	79	230–213	$C_{23}H_{29}KN_2O_5S\cdot H_2O$[d]	55.00	55.81	6.20	6.16
4-CH_3CONH	H	CH_3	B	80	223–226	$C_{19}H_{22}KN_3O_6S$	49.65	49.52	4.83	4.87
4-NO_2	H	CH_3	B	71	202–203	$C_{17}H_{18}KN_3O_7S$	45.63	45.46	4.05	4.19
4-NO_2	3-CF_3	CH_3	B	40	203–205	$C_{18}H_{17}F_3KN_3O_7S$	41.93	41.88	3.33	3.89
3-CH_3	5-CH_3	CH_3	B	60	220–222	$C_{19}H_{23}KN_2O_5S$	53.02	52.92	5.35	5.58
2-Cl	4-C_6H_5	CH_3	B	21	>175	$C_{23}H_{22}ClKN_2O_5S\cdot H_2O$[d,h]	52.01	52.12	4.55	4.50
4-Cl	2-$C_6H_5CH_2$	CH_3	A	42	230–231	$C_{24}H_{24}ClKN_2O_5S\cdot H_2O$[d]	52.92	52.67	4.80	4.75
4-Cl-3,5-xylyl		CH_3	B	31	210–213	$C_{19}H_{22}ClKN_2O_5S$	49.07	48.78	4.77	4.90
4-CH_3O	2-C_6H_5CO	CH_3	A	63	>105	$C_{25}H_{25}KN_2O_7S\cdot H_2O$[d]	54.28	54.90	4.90	4.67

[a] The decomposition point recorded refers to the temperature at which complete decomposition, with evolution of gases, occurred. In all cases darkening and shrinking started at about 10–20° below the complete decomposition point. [b] The starting acid was obtained from Aldrich Chemical Co., Inc. [c] The starting acid was obtained from Eastman Organic Chemicals. [d] Attempt to eliminate the water of crystallization by the usual procedure of heating *in vacuo* at 110° resulted in a more deep-seated decomposition. However, the presence of water was demonstrated by the Karl Fischer water determination method, although the results obtained were low, probably due to the insolubility of these potassium salts in the analytical medium. [e] Calcd.: N, 6.98. Found: N, 7.15. [f] Calcd.: N, 5.73; S, 6.55; Cl, 14.49. Found: N, 5.87; S, 6.16; Cl, 14.50. [g] Calcd.: N, 5.00. Found: N, 5.18. [h] Calcd.: N, 5.28. Found: N, 5.25.

acid was sterically hindered. However, the compounds obtained by method B were found to be easier to purify. No attempt was made to determine the conditions necessary for optimum yields. In many cases where the potassium salt was washed with dry acetone immediately after filtration, no further recrystallization was necessary since the material obtained was found to be analytically pure. In those cases where purification was necessary, recrystallizations from acetone–water or butanol–water were carried out.

(A) **Potassium (DL-α-Phenoxyethyl)-penicillin.**—To a cooled and stirred solution of 54 g. (0.25 mole) of 6-APA in 1.2 liters of water containing 105 g. (1.25 moles) of sodium bicarbonate, a solution of 60 g. (0.325 mole) of DL-α-phenoxypropionyl chloride in 100 ml. of acetone was added in 1 minute. The resulting mixture was stirred vigorously during 20 minutes while the temperature was kept at 10 to 15°. The clear solution was extracted twice with 300-ml. portions of methyl isobutyl ketone (MIBK), the organic extracts being discarded. The clear aqueous solution was covered with 500 ml. of MIBK, cooled to 5–10° and acidified to *p*H 2 with a cold 5 *M* sulfuric acid solution. The MIBK extract was separated, washed with cold water, and dried for 10 minutes over anhydrous sodium sulfate. After filtration, 100 ml. of a 50% solution of potassium 2-ethylhexanoate in butanol was added. The white crystalline material which separated almost immediately was collected by filtration, washed on the filter with a little dry acetone and dried. There was obtained 76 g. (76%) of colorless crystals which decomposed at 230–232°; see Table II for the analysis.

(B) **Potassium [DL-α-(4-Acetamidophenoxy)-ethyl]-penicillin.**—An anhydrous solution of 31.8 g. (0.15 mole) of DL-α-(4-acetamidophenoxy)-propionic acid and 21.2 ml. (0.15 mole) of triethylamine in 330 ml. of dioxane and 65 ml. of acetone was cooled to 0°. To this stirred solution was added dropwise 19.7 ml. (0.15 mole) of isobutyl chloroformate, while the temperature was maintained at 0–4°. The resulting mixture was stirred at 0° for 45 minutes. A cooled solution prepared from 21.6 g. (0.1 mole) of 6-APA, 14.1 ml. (0.1 mole) of triethylamine and 30 ml. of water was added all at once to the mixed anhydride solution. After stirring vigorously at 5 to 8° for one hour, while carbon dioxide was evolved rapidly, a solution of 10.9 g. (0.13 mole) of sodium bicarbonate in 700 ml. of ice-water was then added. The resulting clear solution was extracted twice with 550-ml. portions of ether, the combined ethereal extracts being discarded. The aqueous solution was acidified to *p*H 2 with 63 ml. of 6 *N* sulfuric acid and quickly extracted twice with 800-ml. portions of methyl isobutyl ketone. The combined MIBK extracts were washed with 200 ml. of ice-water, dried briefly over anhydrous sodium sulfate, and filtered. On addition of 40 ml. (0.11 mole) of a 50% solution of potassium 2-ethylhexanoate in butanol an oil separated which soon crystallized when 400 ml. of dry acetone was added. The solid weighed 27.7 g. and decomposed at 211–215°. Addition of 1.5 l. of dry acetone and 2 l. of dry ether to the filtrate precipitated an additional 19.4 g. of product which decomposed at 223–226°. The infrared spectra of the two fractions were identical. After one recrystallization from acetone–water the total yield was 80%; see Table II for analysis.

Potassium (D-α-Phenoxyethyl)-penicillin.—To a stirred, cooled solution of 16.6 g. (0.1 mole) of *d*-α-phenoxypropionic acid in 100 ml. of tetrahydrofuran there was added 10.1 g. (0.1 mole) of triethylamine in one portion. The solution was cooled to −15° and 13.6 g. (0.1 mole) of isobutyl chloroformate was added dropwise at a rate which gave a final reaction temperature of −4°. Immediately there was added in one portion a chilled solution of 21.6 g. (0.1 mole) of 6-APA in 35 ml. of water and 15 ml. of triethylamine. The cooling bath was replaced at once by a water-bath at 55° and the reaction temperature rose to 25° within 5 minutes. The solution was stirred for 25 minutes at 25–27°, diluted with 250 ml. of chilled water and extracted twice with ether. It was then layered with ether, chilled, acidified to *p*H 2 with cold 3 *M* sulfuric acid, extracted twice with ether and the combined ether extracts washed with water and dried over anhydrous sodium sulfate for 10 minutes. To the dried ether solution was added 36.4 g. (0.1 mole) of a 50% solution of potassium 2-ethylhexanoate in butanol. The crystalline product was collected and dried to yield 31.7 g. (79%) of material which decomposed at 214–215°. Successive recrystallizations to constant optical rotation from water–butanol and water–acetone gave 17 g. (42%) of material which decomposed at 234.5–235°. This product had a purity of 99.2% by a phase-solubility assay; $[\alpha]^{20}D$ +252° (*c* 1 in water).

Anal. Calcd. for $C_{17}H_{19}KN_2O_5S$: C, 50.75; H, 4.78; N, 6.98. Found: C, 50.88; H, 4.82; N, 6.94.

Potassium (L-α-Phenoxyethyl)-penicillin.—A solution was prepared by mixing 8.3 g. (0.05 mole) of *l*-α-phenoxypropionic acid, 40 ml. of dry *p*-dioxane, 20 ml. of dry acetone and 8 ml. of triethylamine. To this stirred and cooled solution (*ca.* 0°) was added dropwise, during 10–15 minutes, 6.8 g. (0.05 mole) of isobutyl chloroformate in 10 ml. of *p*-dioxane while the temperature was maintained below 10°. After the addition was completed the mixture was stirred and cooled during 10 minutes, after which time a solution of 10.8 g. (0.05 mole) of 6-APA in 50 ml. of water and 8 ml. of triethylamine was added rapidly. The resulting solution was stirred 15 minutes at *ca.* 10° and then 2 hours at room temperature. After dilution with an equal volume of water the reaction mixture was extracted twice with 100-ml. portions of ether, the ethereal extracts being discarded. The clear aqueous solution was covered with 150 ml. of ether, cooled to 10°, and acidified to *p*H 2 with a cold 5 *M* sulfuric acid solution. The ethereal solution was separated, washed with cold water and dried for 10 minutes over anhydrous sodium sulfate. After filtration 25 ml. of a 50% solution of potassium 2-ethylhexanoate in butanol was added. The white crystalline material which separated was collected by filtration and recrystallized once from 10% aqueous butanol and once again from 10% aqueous acetone. This procedure afforded 9.5 g. (47%) of pure potassium (L-α-phenoxyethyl)-penicillin which decomposed at 238–239°, $[\alpha]^{24}D$ +218° (*c* 1 in water).

Anal. Calcd. for $C_{17}H_{19}KN_2O_5S$: C, 50.75; H, 4.78; N, 6.98. Found: C, 50.92; H, 4.97; N, 6.93.

The same material could be obtained by extensive recrystallizations of potassium (DL-α-phenoxyethyl)-penicillin from a butanol–water mixture. The product obtained in each case had a purity of 98.6–99.8% as determined by the phase-solubility method.[14]

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[CONTRIBUTION FROM THE WILLIAM ALBERT NOYES LABORATORY OF CHEMISTRY, UNIVERSITY OF ILLINOIS, AND THE INSTITUTE OF MICROBIOLOGY, RUTGERS, THE STATE UNIVERSITY]

Chemistry of the Neomycins. V. Differentiation of the Neomycin Complex. Identity of Framycetin and Neomycin B. Compounds Obtained from Methyl Neobiosaminide B

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Neomycin preparations as their N-acetyl derivatives could be resolved qualitatively and quantitatively by paper and column chromatographic techniques. Framycetin thus has been shown to be neomycin B admixed with small quantities of neomycin C. This conclusion was further supported on comparing the products obtained from framycetin and neomycin B by selective chemical degradations of their methyl neobiosaminide moieties. These degradation reactions are outlined in detail.

Soon after the discovery of neomycin by Waksman and Lechevalier,[1] in 1949, it became apparent that the antibacterial activity of the *Streptomyces fradiae* fermentation broth was not due to a single antibiotic but to a number of active substances entitled[2] the "neomycin complex." At the present time there is general agreement concerning the production by *S. fradiae* of two isomeric compounds called neomycin B and neomycin C.[3,4] A third substance, originally called neomycin A[5] but now termed neamine,[6,7,8] is known to arise as a hydrolytic cleavage product of neomycins B and C. The antibiotics catenulin,[9] kanamycin[10,11,12] and paromomycin[13] may also be considered as antibiotics somewhat related to but distinctly different from neomycins B and C.

Aside from these different antibiotics, several other antibiotic mixtures, apparently belonging to the neomycin BC group, also have been reported. Streptothricins BI and BII[14] were shown[15] to be identical with neomycins B and C, respectively.

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