PATENT SPECIFICATION

716,556

Date of Application and filing Complete Specification: July 2, 1951.

No. 15582/51.

Application made in United States of America on July 15, 1950.

Complete Specification Published: Oct. 6, 1954.

Index at acceptance:—Classes 2(3), AA1C(1D1: 2A), AA2C1, C1B2, C2A(1: 2: 3: 5: 7: 9: 13), C2B3(A2: B: D), C2B3G(1: 7: 8), C2B5, C2R(18: 20); and 81(1), B11B2(D: L: N: R: T: Z).

COMPLETE SPECIFICATION

Improvements in Salts of Penicillin

We, AMERICAN HOME PRODUCTS CORPORATION, a Corporation organized and existing under the laws of the State of Delaware, United States of America, of 22, East 40th Street, New York 16, New York, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new salts of penicillin and N-substituted diamines.

It has been found that certain substituted alkylene diamines are useful for separating penicillin from aqueous solutions by forming a salt therewith. Many of these salts are substantially insoluble or, at most, sparingly soluble in an aqueous medium. One is thus able to isolate and, if desired, to purify penicillin since it has been found that the latter can be almost quantitatively removed from its solution by the chemical addition of two molecules of penicillin to these particular diamines.

It has further been discovered that these substantially insoluble, or sparingly soluble, salts of penicillin are valuable from a pharmacological aspect since they are relatively non-toxic and, when in contact with body fluids, slowly release penicillin for utilization in combatting bacterial infections. A prolonged antibiotic effect can thus be achieved without difficulty. By the use of these new penicillin salts, one greatly reduces the number of injections required to sustain the desired blood level concentration of penicillin.

According to the present invention new salts of penicillin and N-substituted diamines are provided, said N-substituted diamines having the general formula

$$\begin{matrix} R \\ \\ R_1 \end{matrix} \!\!>\!N\!-\!Y\!-\!NHR_2$$

wherein R represents an aliphatic, alicyclic, araliphatic, aromatic or heterocyclic group which may or may not have substituents, R_1 represents a hydrogen atom or an aliphatic, alicyclic, araliphatic, aromatic or heterocyclic group which may or may not have substituents, R_2 represents a hydrogen atom or, when R_1 is hydrogen, an aliphatic, alicyclic, araliphatic, aromatic or heterocyclic group which may or may not have substituents, and Y represents an alkylene radical having from 2 to 12 carbon atoms.

The process, according to the invention, for making such salts of penicillin comprises bringing penicillin as such into contact with an N-substituent diamine or, alternatively, bringing a salt of penicillin into contact with a salt of an N-substituted diamine, said N-substituted diamine in either case having the general formula given above. This process may be carried out by mixing an aqueous solution of a salt of penicillin with an aqueous solution of a salt of an N-substituted diamine having the general formula given above and thereafter separating from the aqueous reaction mixture the salt of penicillin with said N-substituted diamine.

Mono-salts can be formed by the combination of one molecule of penicillin with one molecule of the N-substituted diamine or di-salts may be formed by the combination of two molecules of penicillin with one molecule of the N-substituted diamine, the latter salts being preferred.

We have observed that on addition of an aqueous solution of sodium penicillin to an aqueous solution of the N-substituted diamine salt, an immediate precipitate forms which redissolves on shaking. On further addition of sodium penicillin solution, a permanent precipitate forms which is the di-salt. The penicillin salts can also be prepared by adding non-aqueous solutions of free penicillin to non-

aqueous solutions of the N-substituted diamines by using suitable solvents such as ether, acetone, ethyl acetate or amyl acetate.

When salts of the N-substituted diamine are employed, inorganic acids may be used in forming such salts from the substituted alkylene diamine free bases. Carboxylic acid salts are particularly useful. As examples of useful inorganic acids may be mentioned hydrochloric, sulphuric, phosphoric, nitric or hydrobromic acids. Organic acids which have proved useful are acetic, lactic, succinic, malic, aconitic, aminoacetic and tartaric acids. While others will form suitable salts, those organic compounds mentioned will form highly desirable soluble salts whose solubility in water exceeds about 5 grams per 100 cc. of solution at about 30° C.

The useful diamines are those falling within the following general formulæ: $R—NH—Y—NH_2$; $R—NH—Y—NH—R_2$; and $RR_1N—Y—NH_2$ where R, R_1, and R_2 represent either aliphatic, aromatic, araliphatic, alicyclic or heterocyclic radicals, with or without substituents on the rings, and Y represents an alkylene radical having from 2 to 12 carbon atoms.

Representative of important alkylene diamines useful in the isolation of penicillin salts are unsubstituted N,N' - dibenzyl ethylene diamine and those aralkyl compounds having non-interfering substituents on the ring. These substituents, which may also be on the alicyclic and heterocyclic rings as well as on the alkyl chains, may comprise one or more alkyl, alkoxy, halogen, nitro, amino, oxo or hydroxy substituents. As a matter of fact, no substituent has been found which interferes with the reaction of penicillin salts with salts of N:N'-di-substituted secondary ethylene diamines or, indeed, with salts of any of the N-substituted diamines of the general formula given above.

The N:N'-di-substituted alkylene diamines may be prepared, for example from an aldehyde or ketone which may, if desired, be dissolved in a solvent such as alcohol or benzene. To the aldehyde or ketone is added an alkylene diamine, generally in aqueous solution. The reaction product, which is a di-imine, is isolated and is then hydrogenated by catalytic reduction or by a metal-alcohol or metal-acid combination.

The N:N'-di-substituted alkylene diamines can alternatively be made by the reaction of an alkylene dihalide, such as the dibromide, dichloride or di-iodide, with the appropriate amine.

Substituted ethylene diamine salts may also be prepared by interacting approximately equivalent quantities of a salt of ethylene diamine, such as the dihydrochloride, dihydrobromide, dinitrate, diformate, or diacetate, with formaldehyde either in the form of its aqueous solution or as the solid polymeric form, paraformaldehyde, and with a compound containing an active hydrogen such as the alpha-hydrogen of thiophenes, picolines, quinaldines, phenols, aliphatic nitro-compounds, organic acids and esters, and open-chain and cyclic aldehydes and ketones in a solvent such as water, alcohol or other organic solvents or mixtures thereof, resulting in an N:N'-di-substituted ethylene diamine salt which may be transposed into the free base by alkali treatment.

Substituted ethylene diamines or salts thereof may be prepared also by interacting ethylene diamine or the diformate thereof with an aldehyde or ketone and formic acid in formic acid as a solvent. This method is now improved by the use of ethylene diamine diformate, a new salt of ethylene diamine, and replacing the formic acid solvent by ethanol, other alcohols, other organic solvents or water. Both N-substituted- and N:N'-disubstituted-ethylene diamines result from the reaction, and either one may be selected to be the major product by suitably altering the reaction period and the proportions of reactants; equimolar amounts of aldehyde or ketone and ethylene diamine diformate favour N-substituted ethylene diamines, and doubling the amount of aldehyde or ketone and increasing the reaction period favour N:N'-disubstituted ethylene diamines.

Ethylene diamine diformate and its use in the preparation of N-substituted ethylene diamines by reaction with an aldehyde or ketone in a solvent and hydrolysis of the reaction product or by reaction with formaldehyde and a compound containing a reactive hydrogen atom are claimed in our co-pending Application No. 28136/53 (Serial No. 716,598).

The substituted diamine may also be prepared by reacting an aliphatic, araliphatic, aromatic or heterocyclic halide with an alkylene diamine to form the N:N'-disubstituted alkylene diamine in the form of its halide salt, treating the latter with an alkali and extracting the substituted alkylene diamine in the form of a free base. This free base may be converted into a carboxylic or other acid salt by reaction with an aliphatic carboxylic or other acid or acid anhydride.

For the preparation of a penicillin salt, we prefer to use the substituted alkylene diamine in the form of a salt rather than in the form of the free base. The salts may be formed from the free base by dissolving the latter in a solvent such as ether, to which is added the proper acid, depending on the particular salt desired.

The salts formed may be either mono-salts or di-salts depending on the amounts of base and acid used. Thus, if one equivalent of acid and one mol. of base were used, the mono-salt would be formed. On the other hand, if two equivalents, or an excess, of acid, are used with one mol. of the diamine, the di-salt would be

aqueous solutions of the N-substituted diamines by using suitable solvents such as ether, acetone, ethyl acetate or amyl acetate.

When salts of the N-substituted diamine are employed, inorganic acids may be used in forming such salts from the substituted alkylene diamine free bases. Carboxylic acid salts are particularly useful. As examples of useful inorganic acids may be mentioned hydrochloric, sulphuric, phosphoric, nitric or hydrobromic acids. Organic acids which have proved useful are acetic, lactic, succinic, malic, aconitic, aminoacetic and tartaric acids. While others will form suitable salts, those organic compounds mentioned will form highly desirable soluble salts whose solubility in water exceeds about 5 grams per 100 cc. of solution at about 30° C.

The useful diamines are those falling within the following general formulæ:
$R—NH—Y—NH_2$; $R—NH—Y—NH—R_2$; and $RR_1N—Y—NH_2$ where R, R_1, and R_2 represent either aliphatic, aromatic, araliphatic, alicyclic or heterocyclic radicals, with or without substituents on the rings, and Y represents an alkylene radical having from 2 to 12 carbon atoms.

Representative of important alkylene diamines useful in the isolation of penicillin salts are unsubstituted N,N¹ - dibenzyl ethylene diamine and those aralkyl compounds having non-interfering substituents on the ring. These substituents, which may also be on the alicyclic and heterocyclic rings as well as on the alkyl chains, may comprise one or more alkyl, alkoxy, halogen, nitro, amino, oxo or hydroxy substituents. As a matter of fact, no substituent has been found which interferes with the reaction of penicillin salts with salts of N:N¹-di-substituted secondary ethylene diamines or, indeed, with salts of any of the N-substituted diamines of the general formula given above.

The N:N¹-di-substituted alkylene diamines may be prepared, for example from an aldehyde or ketone which may, if desired, be dissolved in a solvent such as alcohol or benzene. To the aldehyde or ketone is added an alkylene diamine, generally in aqueous solution. The reaction product, which is a di-imine, is isolated and is then hydrogenated by catalytic reduction or by a metal-alcohol or metal-acid combination.

The N:N¹-di-substituted alkylene diamines can alternatively be made by the reaction of an alkylene dihalide, such as the dibromide, dichloride or di-iodide, with the appropriate amine.

Substituted ethylene diamine salts may also be prepared by interacting approximately equivalent quantities of a salt of ethylene diamine, such as the dihydrochloride, dihydrobromide, dinitrate, diformate, or diacetate, with formaldehyde either in the form of its aqueous solution or as the solid polymeric form, paraformaldehyde, and with a compound containing an active hydrogen such as the alpha-hydrogen of thiophenes, picolines, quinaldines, phenols, aliphatic nitro-compounds, organic acids and esters, and open-chain and cyclic aldehydes and ketones in a solvent such as water, alcohol or other organic solvents or mixtures thereof, resulting in an N:N¹-di-substituted ethylene diamine salt which may be transposed into the free base by alkali treatment.

Substituted ethylene diamines or salts thereof may be prepared also by interacting ethylene diamine or the diformate thereof with an aldehyde or ketone and formic acid in formic acid as a solvent. This method is now improved by the use of ethylene diamine diformate, a new salt of ethylene diamine, and replacing the formic acid solvent by ethanol, other alcohols, other organic solvents or water. Both N-substituted- and N:N¹-disubstituted-ethylene diamines result from the reaction, and either one may be selected to be the major product by suitably altering the reaction period and the proportions of reactants; equimolar amounts of aldehyde or ketone and ethylene diamine diformate favour N-substituted ethylene diamines, and doubling the amount of aldehyde or ketone and increasing the reaction period favour N:N¹-disubstituted ethylene diamines.

Ethylene diamine diformate and its use in the preparation of N-substituted ethylene diamines by reaction with an aldehyde or ketone in a solvent and hydrolysis of the reaction product or by reaction with formaldehyde and a compound containing a reactive hydrogen atom are claimed in our co-pending Application No. 28136/53 (Serial No. 716,598).

The substituted diamine may also be prepared by reacting an aliphatic, araliphatic, aromatic or heterocyclic halide with an alkylene diamine to form the N:N¹-disubstituted alkylene diamine in the form of its halide salt, treating the latter with an alkali and extracting the substituted alkylene diamine in the form of a free base. This free base may be converted into a carboxylic or other acid salt by reaction with an aliphatic carboxylic or other acid or acid anhydride.

For the preparation of a penicillin salt, we prefer to use the substituted alkylene diamine in the form of a salt rather than in the form of the free base. The salts may be formed from the free base by dissolving the latter in a solvent such as ether, to which is added the proper acid, depending on the particular salt desired.

The salts formed may be either mono-salts or di-salts depending on the amounts of base and acid used. Thus, if one equivalent of acid and one mol. of base were used, the mono-salt would be formed. On the other hand, if two equivalents, or an excess, of acid, are used with one mol, of the diamine, the di-salt would be

* C L M S *

formed. For making penicillin salts from the diamine salts, one would generally desire the di-salt instead of the mono-salt since two mols. of penicillin can be combined when using the former, whereas only one mol. of penicillin can be combined when the mono-salt is used.

In preparing the penicillin salts, the diamine salts are generally dissolved in an aqueous medium and reacted with an aqueous penicillin salt solution. Where the reactions are to take place in an aqueous solution, it has been found advantageous to use relatively soluble diamine salts in preparing the penicillin salts thereof since less liquid volume is handled and therefore losses are likely to be less than if relatively insoluble salts were used. While salts made with the inorganic acids can be used in preparing the penicillin compounds, it has been found that their solubility is quite low and well below 5% on a weight basis. Consequently, the diacetates, having a solubility in the neighbourhood of about 10% or higher have been found particularly effective where it is desired to handle relative low liquid volumes and therefore high concentrations.

Any of the known water-soluble penicillin salts may be used for reaction with the N-substituted diamine salts. Thus, one may use the alkali or alkaline earth metal salts of penicillin G, dihydro F, X, or penicillin K for combination with the selected diamine. However, the more preferred penicillin salts are the sodium or potassium salts of penicillin G. One or two molecules of any penicillin above indicated will unite with one molecule of the diamine, depending on the use of the mono or the di-salt.

The following examples are given for specific illustrations of the present invention. Examples 1 to 27 illustrate the preparation of the substituted diamines and salts thereof and Examples 28 to 33 illustrate the preparation of the new penicillin salts. The temperatures in these Examples are given in degrees Centigrade.

EXAMPLE 1.

PREP. NO. 1 OF N:N'-DIBENZYLETHYLENEDIAMINE AND SALTS THEREOF.

N:N^1-dibenzalethylenediamine was first prepared by condensing benzaldehyde with ethylene diamine. The dibenzalethylenediamine (23.6 g.) was dissolved in 100 cc. methanol containing 0.5 g. Adams' platinum catalyst and hydrogenated at 50 lbs. pressure. Slightly more than the theoretical amount of hydrogen was absorbed in 45 minutes. The catalyst was filtered off, washed with methanol, the filtrate concentrated and the residue fractionated. Nearly all of the material distilled at 160° C. and 0.75 mm., n_D^{20} 1.5621.

With Raney nickel catalyst at atmospheric pressure little hydrogen absorption occurred; however, under elevated pressure hydrogenation went to completion but was much slower than with platinum. The diacetate was prepared by dissolving 440 g. of the base in about 3 litres of 95% alcohol and slowly adding 220 g. of glacial acetic acid. After standing some time the crystalline solid was filtered off and recrystallized from hot alcohol. Long, white needles, m.p. 110—112°.

Calcd. for $C_{16}H_{20}N_2 . 2CH_3COOH$: C, 66.7; H, 7.78; N, 7.78. Found: C, 66.98; H, 7.63; N, 8.38. Solubility 252.9 g. per litre of solution at 30.4° in water. The dihydrobromide was obtained by adding hydrobromic acid to an aqueous solution of the acetate. Large colourless plates, recrystallized from water, m.p. 300° (bar). Calcd. for $C_{16}H_{20}N_2 . 2HBr$: N, 6.97; Br, 39.8. Found: N, 6.9; Br, 39.5. Solubility 30.0 g. per litre of solution in water at 30.4°.

The dihydrochloride was obtained from the acetate by addition of hydrochloric acid and recrystallized from water. Large white plates, m.p. 294° (bar). Calcd. for $C_{16}H_{20}N_2 . 2HCl$: N, 8.97; Cl, 22.4. Found: N, 8.8; Cl, 22.4. Solubility 23.9 g. per litre of solution in water at 30.4°.

The dinitrate was obtained by adding nitric acid to a solution of the acetate. Large white plates, m.p. 274° (bar). Calcd. for $C_{16}H_{20}N_2 . 2HNO_3$: N, 15.3. Found: N, 14.9. Solubility 9.04 g. per litre of solution at 30.4°.

The phosphate was obtained by adding phosphoric acid to a solution of the acetate and recrystallized from dilute alcohol. White prisms, m.p. 232° (bar). Calcd. for $C_{16}H_{20}H_2 . 2H_3PO$: N, 6.42; P, 14.2. Found: N, 5.45; P, 13.9. Solubility 38.6 g. per litre of solution at 30.4°.

The sulphate was obtained by addition of sulphuric acid to a solution of the acetate and recrystallization from alcohol. White plates, m.p. 247—250°. Calcd. for $C_{16}H_{20}N_2H_2SO_4$: C, 56.8; N, 8.29; H, 6.51; S, 9.47. Found: C, 56.37; H, 6.43; N, 7.99; S, 9.85. Solubility 15.8 g. per litre of solution at 30.4°.

The thiocyanate was prepared by addition of a solution of sodium thiocyanate to the acetate and recrystallization from alcohol. White needle-like plates, m.p. 212° (bar). Calcd. for $C_{16}H_{20}N_2 . 2HCNS$; N, 15.63; Found: N, 15.4.

The salicylate was obtained by addition of a solution of sodium salicylate to a solution of the acetate and recrystallization from water. White crystals, m.p. 85° (bar). Calcd. for $C_{16}H_{20}N_2 . 2C_7H_6O_3$, N, 5.42; Found: N, 5.24.

The picrate was obtained from the acetate by addition of a solution of lithium picrate and recrystallization from alcohol, m.p. 211° (bar).

The oxalate, $C_{16}H_{20}N_2 . H_2C_2O_4$, was obtained as colourless needles from alcohol, m.p. 275—6°.

The lactate was prepared by mixing alcoholic solutions of the acid and base. Fine white crystals, m.p. 74°.

The salts of succinic, propionic, malic,

* C L M S *

aconitic, aminoacetic and tartaric acids did not precipitate out on addition of their aqueous sodium salt solutions to the solutions of the acetate indicating that these salts were substantially water-soluble. To obtain these salts, the solutions are evaporated to dryness. A better procedure to obtain a purified product is to dissolve the diamine free base in an organic solvent such as alcohol, acetone or diethyl ether, and add the free acid also in solution in an organic solvent. The desired salt will either precipitate from solution, or the solvent solution may be evaporated to dryness.

Example 2.

Prep. No. 2 of N:N¹-dibenzylethylenediamine and a salt thereof.

Ethylenediamine (15 g., 0.25 mol.) was added dropwise to 100 ml. 98—100% formic acid in a two-necked 500 ml. flask, fitted with an addition tube and reflux condenser with drying tube, and cooled in an ice-bath. After complete addition of the base 53 g. of benzaldehyde (0.5 mol.) was added in one lot. The ice-bath was removed and the flask was heated to the refluxing temperature. The initial rate of carbon dioxide evolution was too rapid to measure. After twenty minutes, the rate was about 100 ml. per minute and decreased rapidly to 8 ml. per minute during one hour. Heating at reflux was continued for 35 hours.

A second, similar experiment, differing from the first only in the substitution of 100 ml. 88—90% formic acid for 100 ml. 98—100% formic acid evolved carbon dioxide at a slightly slower rate.

The two reaction mixtures were combined and most of the excess formic acid was removed under reduced pressure. Hydrochloric acid (200 ml. 6N) was added to the viscous amber residue and the mixture heated under reflux. After 15 minutes, bumping necessitated cooling and filtering to remove crystalline dihydrochloride, which after washing with isopropanol was dried; m.p. circa 300°. The mother liquors were refluxed one hour and cooled, obtaining an additional amount of product, m.p. circa 300°. The filtrate was concentrated *in vacuo* to 100 ml., cooled and made alkaline with 40% NaOH. The supernatant oil was extracted with ether, dried, and fractionated from a still-pot packed with glass wool and heated in a sand-bath at 320°. The first fraction at 106° at 0.6—0.7 mm. was N-benzylethylenediamine (dipicrate, m.p. 222°). The N:N - dibenzylethylenediamine was collected at 177—206° at 0.6—1.0 mm. as a colourless liquid.

Crystalline dihydrochloride was converted to free base with aqueous sodium hydroxide and distilled over sodium hydroxide at 153—158° at 0.5 mm. The colourless distillate solidified to white crystals melting at 24—25°.

Example 3.

Prep. No. 1 of N:N¹-dipiperonylethylenediamine and salts thereof.

Piperonal, 150 g. (1 mol.), was dissolved in 400 cc. ethanol and treated with 35 g. (0.5 mol.) of aqueous ethylenediamine (78%) and allowed to stand a short time (heat is evolved). The resulting white crystalline solid N:N¹-dipiperonylethylenediamine was filtered off, washed with alcohol and dried. Yield is theoretical, m.p. 179—180°.

A mixture of 388 g. (1.2 mols.) of the above compound in 1500 cc. methanol and 1 g. of Adams' platinum catalyst was hydrogenated at 810 lbs./sq.in. until the required amount of hydrogen was taken up. The solution was then concentrated to remove most of the methanol, taken up in water containing acetic acid and hydrochloric acid then added to obtain the slightly soluble hydrochloride which was recrystallized from hot water, m.p. 277—280°. Calcd. for $C_{18}H_{22}O_4N_2Cl_2$: C, 54.0; H, 5.5; N, 7.0; Cl, 17.4. Found: C, 54.39; H, 5.41; N, 7.04; Cl, 17.55.

The hydrochloride was converted to the free base by treatment with strong alkali and extraction with ether. The free base is a white crystalline solid (needles); m.p. 145—8° C.

The diacetate salt, prepared from the free base in ether by addition of glacial acetic acid, is a white crystalline solid, m.p. 133—4°. Calcd. N, 6.25. Found: 6.25, 6.25, 6.44. Calcd. M.W. 448. Found: 448.

The flavianate was obtained in yellow crystals, m.p. 230° dec. (bar). The picrate was obtained from acetone in yellow needles, m.p. 216° dec.

Example 4.

Prep. No. 2 of N:N¹-dipiperonylethylenediamine and a salt thereof.

Ethylene diamine diformate was first prepared by the dropwise addition of 120 ml. crude ethylene-diamine diluted with 100 ml. acetone to a cooled and stirred mixture of 200 ml. 88—90% formic acid and 200 ml. acetone. After complete addition, the precipitated solid was collected on a filter and washed with acetone. The lumpy product was powdered in a mortar to facilitate washing by suspension in acetone. After drying, the product weighed 200 grams. A sample recrystallized from methanol formed white crystals, m.p. 132° (bar). Anal. $C_4H_{12}N_2O_4$: N, 18.42. Found: N, 18.43.

In a 250 ml. flask fitted with a reflux condenser with drying tube, there were mixed 30 grams (0.2 mol.) of piperonal, 15.2 grams (0.1 mol.) of ethylenediamine diformate, and 50 ml. 98—100% formic acid. The reaction mixture was heated to reflux; after 10 minutes the rate of carbon dioxide evolution was 20 ml. per minute, after 54 hours the rate was 0.3 ml. per minute. Most of the excess formic acid was removed under reduced pressure. Then 50 ml. 6N hydrochloric acid were added to the dark residue and the mixture heated on a steam

* C L M S *

cone for two hours. On cooling, dark crystals formed. These were filtered and washed with isopropanol leaving almost white crystals, m.p. 300° (bar). The mother liquors were concentrated to 50 ml. and diluted with 50 ml. water throwing out a dark oil which was removed by benzene extraction. The aqueous phase was diluted with 50 ml. ethanol, heated, treated with charcoal, and, on concentration to circa 75 ml. and cooling, an additional crop of crystals was obtained. Recrystallization from aqueous ethanol and from water did not raise the m.p. of the white, crystalline, dihydrochloride above 300°.

EXAMPLE 5.

PREPARATION OF N:N¹-DIFURFURYLETHYLENEDIAMINE AND SALTS THEREOF.

170 grams of ethylenediamine (78%) was vigorously stirred with 500 cc. benzene in a nitrogen atmosphere while being cooled in ice. Then 454 g. furfural was slowly added and stirred an hour after addition was complete. The benzene layer was removed and dried over solid sodium hydroxide.

Without isolating the above product a portion of the benzene solution representing 200 g. of product was reduced in 3 litres of absolute alcohol with 350 g. of sodium. The alcohol was partially removed, water added and the mixture extracted with ether. The ether layer was then dried, the ether removed and the residue fractionated. The N:N¹-difurfuryl-ethylene diamine distilled at 131—141°/0.15 mm., $n_D^{22.5}$ 1.5183.

The diacetate, prepared by solution of the base in ether and adding acetic acid followed by recrystallization of the filtered solid from ethyl acetate, formed white needles which melted unsharply about 100—104°. Calcd. for $C_{16}H_{24}N_2O_6$: C, 56.5; H, 7.06; N, 8.23. Found: C, 56.84; H, 7.02; N, 8.32.

The picrate was obtained as large golden-yellow prisms of m.p. 165—167.5° or as large, clear dark-yellow plates of m.p. 178° dec. (Bar.). Calcd. for $C_{12}H_{16}O_2N_2 \cdot 2C_6H_3O_7N_3$: C, 42.5; H, 3.24. Found: C, 42.73; H, 3.11.

EXAMPLE 6.

PREPARATION OF N:N¹-BIS-(2-HEPTYL)-ETHYLENEDIAMINE AND SALTS THEREOF.

131.1 g. of 2-heptylamine and 214 g. of ethylene bromide were warmed on the steam-bath yielding a brownish-red syrup. On stirring the syrup with ether it slowly dissolved and precipitated a white solid. The solid was filtered off and recrystallized from methanol, m.p. 282°. Calcd. for $C_{16}H_{36}N_2 \cdot 2HBr$: N, 6.7; Br, 38.2. Found: N, 6.09; Br, 36.0. The picrate had m.p. 150°.

The hydrobromide was treated with sodium hydroxide solution yielding an oily upper layer which was extracted with ether. The ether layer was dried over solid sodium hydroxide, the ether removed and the residue fractionated. The product had b.p. 125—127° at 2 mm., n_D^{20} 1.4498. Calcd. for $C_{16}H_{36}N_2$: C, 75.0; H, 14.07; N, 10.9. Found: C, 73.41, 73.05; H, 12.50, 12.36; N, 10.58.

EXAMPLE 7.

PREPARATION OF N:N¹-BIS-(GAMMA-PHENYL-PROPYL)-ETHYLENEDIAMINE AND A SALT THEREOF.

44 g. of cinnamaldehyde was slowly added to 15 cc. ethylenediamine-water azeotrope. In a short time the reaction mixture solidified. Benzene was added to dissolve the solid and the water of reaction removed by drying over calcium chloride. After cooling and addition of some ether, light tan crystals formed which were filtered off and air-dried. The solid in methanol was hydrogenated over Adams' platinum catalyst and after 12 lbs. gauge (0.15 mol) hydrogen was absorbed, the methanol was removed and the residue was fractionated. The first fraction was gamma-phenyl-propanol, b.p. 101—108°/1 mm., n_D^{20} 1.5309. The second fraction was N:N¹-bis-(gamma-phenylpropyl)-ethylenediamine, b.p. 155—162°/0.25 mm., n_D^{20} 1.5551. The dihydrochloride decomposed between 260 and 270°, m.p. 274° (bar). Calcd. for $C_{20}H_{28}N_2 \cdot 2HCl$: C, 65.0; H, 8.2; N, 7.6. Found: C, 66.43; H, 8.18; N, 7.3.

EXAMPLE 8.

PREPARATION OF N:N¹-DICYCLOHEXYL-ETHYLENEDIAMINE AND SALTS THEREOF.

200 g. of cyclohexylamine and 50 g. ethylene chloride were warmed several hours on the steam-bath until the material became solid. Hot water was added and the mixture was made strongly alkaline. The oily layer began to crystallize as soon as it cooled a few degrees, and was separated from the liquid as large colourless crystals, m.p. 99°. On recrystallization from dilute alcohol the solid melted at 82°, yield 77.5 g. Calcd. for $C_{14}H_{28}N_2 \cdot H_2O$: N, 11.6. Found: N, 10.91.

The following salts were obtained by adding the corresponding acids to an alcohol solution of the base until no further precipitation occurred and recrystallizing the crude products from water:

Dihydrochloride, white plates, m.p. 315° (bar). Calcd. for $C_{14}H_{28}N_2 \cdot 2HCl$: N, 9.43; Found: N, 9.14.

Dihydrobromide, white plates, m.p. 339 dec. (bar).

Dinitrate, small white crystals, dec. 230° (bar).

Diflavianate, small yellow crystals, dec. 312° (bar).

Phosphate, white plates, m.p. 313° (bar).

Picrate, yellow crystals, m.p. 218° (bar).

Sulphate, large white plates, m. above 350° (bar).

The acetate was prepared in ether from the base and acetic acid as large white crystals, m.p. 144°.

The acetate in water solution on treatment with potassium cyanate yielded the corresponding bis-urea, small white crystals from dilute alcohol, m.p. 248° (bar).

* C L M S *

The dinitroso compound was prepared from the acetate and sodium nitrite in water. White crystalline flakes, m.p. 130—1° (bar).

EXAMPLE 9.

PREPARATION OF N:N¹-BIS-(4-METHYL-2-PENTYL)-ETHYLENEDIAMINE.

200 g. of 2-amino-4-methylpentane and 94 g. of ethylene bromide were warmed on the steam-bath. The reaction was quite vigorous. The material was treated with a strong solution of sodium hydroxide and the dark oily layer extracted with ether. The ether layer was dried over alkali, the ether removed and the residue fractionated. After taking off all material up to a bath temperature of 200° at atmospheric pressure the remaining material comprising the desired product distilled between 95 and 97° at 1 mm., n_D^{25} 1.4419.

EXAMPLE 10.

PREPARATION OF N:N¹ - BIS - (3:5:5 - TRIMETHYLHEXYL)-ETHYLENEDIAMINE AND ITS SALTS.

286 g. of 3:5:5-trimethylhexylamine and 50 g. ethylene chloride were warmed on the steam-bath for several days after which time the reaction mass was very viscous and jellied on cooling. Water was added followed by a strong solution of sodium hydroxide. This was extracted with ether, the extract dried over solid sodium hydroxide, the ether removed and the residue fractionated. The fractions boiling between 123 and 200° at 0.06 to 0.08 mm. were combined and refractionated. Most of the material distilled 149 to 154° at 1.2 mm. and had $n_D^{22.5}$ 1.4548.

The acetate was prepared by adding acetic acid to an ethereal solution of the base and recrystallizing from ethyl acetate, small white flakes, m.p. 105°. Calcd. for $C_{20}H_{44}N_2 . 2CH_3COOH$: N, 6.48. Found: N, 6.40.

The hydrobromide was prepared by adding hydrobromic acid to an alcoholic solution of the base and recrystallizing the product from dilute alcohol, fine white crystals, m.p. 246° (bar). Calcd. for $C_{20}H_{44}N_2 . 2HBr$: N, 5.36; Br, 30.6. Found: N, 5.8; Br, 33.15.

The hydrochloride was prepared in the same manner as the hydrobromide. Larger white plates, m.p. 256° (bar).

The nitrate was obtained as above. Large white flakes, m.p. 214° (bar). Solubility 2.14 mg./ml. at 30°.

The phosphate obtained as above was recrystallized from dilute alcohol.

The sulphate was recrystallized from water. White crystals, m.p. 290° (bar). dec. Calcd. for $C_{20}H_{44}N_2O_4S$: N, 6.83. Found: N, 6.31.

H44

EXAMPLE 11.

PREPARATION OF N:N¹-BIS-(*p*-CHLOROBENZYL)-ETHYLENEDIAMINE AND ITS SALTS.

25 g. of *p*-chlorobenzyl chloride, 5 g. of nearly anhydrous ethylenediamine and 50 cc. of xylene were warmed to 100° for several hours. A solid gradually formed. On cooling the reaction mass, water was added followed by a strong solution of sodium hydroxide. The oily layer was extracted with ether and the ether layer was then extracted with dilute acetic acid. The acid layer was made alkaline and extracted with ether. The ether layer was dried over magnesium sulphate and filtered through carbon. Glacial acetic acid was then added to the ether solution as long as a precipitate formed which was then removed and recrystallized from ethyl acetate to give fine white crystals, m.p. 126° (bar). Calcd. for $C_{16}H_{18}N_2Cl_2 . 2CH_3COOH$: N, 6.5. Found: N, 7.21.

The picrate was obtained from an aqueous solution of the acetate by addition of lithium picrate. Fine yellow crystals, m.p. 193° (bar).

EXAMPLE 12.

PREPARATION OF N:N¹-BIS-(2:4-DICHLOROBENZYL)-ETHYLENEDIAMINE AND ITS SALT.

25 g. of 2:4-dichlorobenzyl chloride, 4 g. of nearly dry ethylenediamine and 50 cc. of xylene were heated at 100—130° overnight. Water and alkali were added to the cooled reaction mass, which was extracted with ether and the ether layer extracted with dilute acetic acid. The acid extract was made alkaline and extracted with ether. The ether layer was dried over magnesium sulphate and glacial acetic acid was added to the filtered ether extract. After allowing the ether to evaporate a syrupy residue remained which crystallized very slowly. The picrate was obtained as yellow crystals, m.p. 183° (bar). dec.

EXAMPLE 13.

PREPARATION OF N:N¹-BIS-(*p*-NITROBENZYL)-ETHYLENEDIAMINE AND A SALT THEREOF.

In a 50 ml. flask fitted with a reflux condenser and drying tube, there were placed 3.0 g. (0.02 mol.) of *p*-nitro-benzaldehyde, 1.5 g. (0.01 mol.) of ethylenediamine diformate and 10 ml. 98—100% formic acid. After heating for 30 hours, 385 ml. (84%) of evolved carbon dioxide were collected. Ten ml. of 6N hydrochloric acid were added and the mixture was refluxed for 1½ hours. When diluted with 10 ml. water and cooled, light tan crystals separated. The dihydrochloride was collected on a filter, washed with cold water and dried, 2.5 g. (62%). A portion, recrystallized from aqueous ethanol (charcoal), afforded white crystals, m.p. 290° (bar). Calcd. for $C_{16}H_{20}N_4O_4Cl_2$: C, 47.66; H, 5.00; N, 13.89; Cl, 17.54. Found: C, 47.73; H, 5.02; N, 13.87; Cl, 17.38.

The free base may also be made by reacting *p*-nitrobenzyl-chloride and ethylenediamine in xylene in the same manner as disclosed in the above examples. The various salts may be made as already disclosed. The *o*- and the *m*-nitrobenzyl compounds may be made by either method starting with the proper aldehyde or halide. The same holds true for the di-nitro compounds.

EXAMPLE 14.

PREPARATION OF N-MONO-(*p*-HYDROXYBENZYL)-ETHYLENEDIAMINE AND ITS SALTS.

Ethylenediamine (30 g., 0.5 mol.) was added dropwise to 150 ml. 98—100% formic acid in a two-necked 500 ml. flask, fitted with an addition tube and a reflux condenser with drying tube, immersed in an ice-bath. After complete addition, 122 g. (1.0 mol.) of *p*-hydroxybenzaldehyde was added all at once, the ice-bath replaced with a heater, and the reaction heated to reflux. After 18 hours, carbon dioxide evolution had decreased to 3.4 ml. per minute. Approximately 65 ml. of formic acid was removed under reduced pressure, and the reddish residue poured into 400 ml. 6 N hydrochloric acid and stirred and refluxed one hour. After cooling and filtration to remove a reddish scum, the filtrate was diluted with acetone, precipitating 25 g. of ethylenediamine dihydrochloride as white crystals, m.p. 310° (bar). The mother liquors were taken to dryness on a steam bath, leaving a black solid which was leached with hot water and filtered (removing colour with superfiltrol). The filtrate was taken to dryness under reduced pressure leaving a light-tan solid residue which was suspended in alcohol and filtered, affording white crystals, m.p. 208°. A sample recrystallized from aqueous alcohol melted at 214° (bar) and proved to be N - mono - (*p*-hydroxybenzyl) ethylene diamine.

The dipicrate was prepared by adding aqueous lithium picrate to an aqueous solution of dihydrochloride, m.p. 214°. The yellow precipitate was crystallized twice from aqueous ethanol, m.p. 142° (bar).

EXAMPLE 15.

PREPARATION OF N:N¹-BIS-(*p*-AMINOBENZYL)-ETHYLENEDIAMINE AND A SALT THEREOF.

N:N¹-bis-(*p*-nitrobenzyl) - ethylenediamine monoacetate (0.43 g., 1.1 millimol), prepared by reacting one mol. equivalent of free base with one mol. of acetic acid, and 0.10 g. platinum oxide were placed in a 50 ml. Erlenmeyer flask with 10 ml. methanol. The flask was attached to a reservoir of hydrogen, flushed with hydrogen and then shaken at room temperature and atmospheric pressure. Reduction was complete in 70 minutes with theoretical uptake of hydrogen. The catalyst was removed by filtration, and addition of 0.4 ml. 6N hydrochloric acid to the filtrate precipitated the dihydrochloride. The white crystals were recrystallized from aqueous methanol, m.p. over 300°.

EXAMPLE 16.

PREPARATION OF N:N¹ - BIS - (*p*-METHOXYBENZYL) - ETHYLENEDIAMINE AND SALTS THEREOF.

Ethylenediamine (30 g., 0.5 mol.) was added dropwise to 150 ml. 98—100% formic acid in a two-necked 500 ml. flask, fitted with an addition tube and reflux condenser with drying tube, immersed in an ice-bath. After complete addition, anisaldehyde (136 g. 1 mol.) was added all at once, the ice-bath replaced by a heater, and the reaction mixture heated to reflux. Evolution of carbon dioxide through the drying tube was occasionally determined by displacement of water. Ten minutes after attaining reflux, the rate was 175 ml. per minute, at one hour 21 ml. per minute, and at 17 hours 3 ml. per minute, at which point heating was discontinued. Excess formic acid was removed under reduced pressure at water-bath temperature. The dark viscous residue was taken up in 800 ml. ethanol and 500 ml. 6N hydrochloric acid, and the mixture stirred and refluxed for six hours. The resulting mixture of solvent and crystals was cooled in an ice-bath, filtered, and the white crystals washed with ethanol. The dried dihydrochloride had an m.p. 278—280°. A portion recrystallized from 50% aqueous alcohol melted 287°. Calcd. for $C_{18}H_{26}N_2O_2Cl_2$: C, 57.90; H, 7.02; N, 7.51; Cl, 19.0. Found: C, 58.41; H, 7.31; N, 7.37; Cl, 19.4.

A dipicrate was prepared from an aqueous solution of dihydrochloride by addition of aqueous lithium picrate. The yellow precipitate was recrystallized first from ethanol and then from aqueous acetone, m.p. 215°. Calcd. for $C_{30}H_{30}N_8O_{16}$: C, 47.50; H, 3.99; N, 14.77. Found: C, 47.65; H, 4.27; N, 14.53.

A dinitrate was prepared by addition of dilute nitric acid to an aqueous solution of the dihydrochloride. The white precipitate was recrystallized from water, m.p. 220° dec. Calcd. for $C_{18}H_{26}N_4O_8$: C, 50.70; H, 6.15; N, 13.14. Found: C, 50.97; H, 6.17; N, 13.44.

EXAMPLE 17.

PREPARATION OF N:N¹-BIS-(2-THENYL)-ETHYLENEDIAMINE AND A SALT THEREOF.

In a 500 ml. three-necked flask, fitted with stirrer, condenser and thermometer, were mixed 42 g. (0.5 mol.) of thiophene, 33 g. (0.25 mol.) ethylenediamine dihydrochloride and 43 ml. of 36% aqueous formaldehyde (0.5 mol.). The mixture was stirred and heated to gradually raise the temperature. At 60° a vigorous reaction began. Heating was stopped and an ice-bath applied to the flask. The internal temperature rose to 73° and the reaction mixture solidified. 200 ml. of 50% aqueous alcohol were added and stirred and the mixture was heated an additional 1½ hours. After cooling, the reaction product was filtered and washed with water. The white product was amorphous and did not dry well, nor could it be crystallized.

It was dissolved in 250 ml. hot water, cooled, and made alkaline with 40% sodium hydroxide. The free base which separated was not very soluble in ether, and was taken up in benzene, dried over sodium hydroxide and obtained as a colourless, viscous oil on removing benzene *in vacuo*. The oil was converted to diacetate by dissolving in 200 ml. ethyl acetate and adding 12 ml. glacial acetic acid. The

precipitated salt was filtered, washed with ethyl acetate and dried, m.p. 84° (Bar).

In essentially the same manner as taught by Example 17, one may also react an alkylene diamine such as ethylenediamine and an acid, as, for example, hydrochloric or sulphuric acid, to form the di-acid salt of the alkylene diamine, and using 0.25 mol. of the di-acid salt together with half a mol. of formaldehyde, half a mol. of the following compounds may be reacted therewith to form the corresponding symmetrical di-substituted alkylene diamines: cyclohexanone, 2-, 3- and 4-methyl-cyclohexanone, 4-methoxy-cyclohexanone, cyclopentanone, 2-methyl-thiophene, isoquinoline, 3-methyl-isoquinoline and quinaldine.

Example 18.

Preparation of N-benzyl-N'-alpha-ethyl-benzyl-ethylenediamine and salts thereof.

Dimethyl benzylaminoacetal (49 g., 0.25 mol.) and 1-phenyl-propylamine (34 g., 0.25 mol.) were mixed in a 500 ml. flask fitted with a reflux condenser with a drying tube. Formic acid (75 ml., 98—100%) was added all at once. A vigorous reaction ensued with darkening, evolution of heat and carbon dioxide. When the initial vigorous reaction had subsided, the mixture was heated. Ten minutes after attaining reflux, the rate of CO_2 evolution was 250 ml. per minute, decreasing to 0.6 ml. per minute in two hours. Excess formic acid was removed under reduced pressure leaving a dark tarry residue to which was added 150 ml. 6N HCl. After heating under reflux for one hour, cooled in ice-bath and made alkaline by addition of 50% aqueous sodium hydroxide. The resulting black supernatant layer was separated, diluted with 400 ml. ether and filtered to remove tar. The filtrate was dried over sodium hydroxide and treated with methanolic hydrochloric acid. The resulting tan precipitate was recrystallized from aqueous isopropanol (charcoal) forming white crystalline dihydrochloride, m.p. over 300°.

A portion recrystallized from aqueous methanol melted at 306° (bar). Calcd. for $C_{18}H_{26}N_2Cl_2$: C, 63.33; H, 7.68; N, 8.21. Found: C, 61.81; H, 6.96; N, 8.28.

The dipicrate was made by addition of aqueous lithium picrate to aqueous dihydrochloride. The yellow crystals were recrystallized from aqueous ethanol. Calcd. for $C_{30}H_{30}N_8O_{14}$: C, 49.59; H, 4.16; N, 15.43. Found: C, 48.26; H, 3.70; N, 15.55.

The dinitrate was made by addition of dilute nitric acid to aqueous dihydrochloride. The white crystals were recrystallized from aqueous ethanol, m.p. 278°. Calcd. for $C_{18}H_{26}N_4O_6$: C, 54.80; H, 6.65; N, 14.20. Found: C, 52.85; H, 5.94; N, 14.92.

Example 19.

Preparation of N:N'-bis-(*p*-methylbenzyl)-ethylenediamine and its salts.

Six g. of *p*-xylylamine, 4.3 g. ethylene bromide and 10 cc. of butanol were refluxed for one hour. On cooling, the solution became solid. The solid was filtered off, washed with butanol and ether, and dried at room temperature. After one crystallization from dilute alcohol it has m.p. 296° (bar).

The salt was then suspended in water and treated with 40% sodium hydroxide solution. The oil was extracted with ether, the ether layer dried over sodium sulphate, filtered and the ether removed. The residue was taken up in a little ether, glacial acetic acid added in sufficient amount to form the diacetate and the solid filtered and recrystallized from ethyl acetate. White crystals, m.p. 116°.

The ortho- or meta-methylbenzyl compounds may be made similarly by starting with *o*- or *m*-xylylamine.

Example 20.

Preparation of 1:10-bis(benzylamino)-decane and its salts.

A solution of 39.4 g. decamethylene iodide and 21.4 g. benzylamine in 200 cc. isopropanol was refluxed for about 4 to 5 hours. On standing overnight a white crystalline solid separated out which was filtered off, washed with ether, and dried yielding 1:10-bis(benzylamino)-decane dihydroiodide, m.p. 238° (bar). More could be obtained from the mother liquor by concentration.

Five grams of the hydroiodide was suspended in water and treated with 40% sodium hydroxide solution, extracted with ether, the ether evaporated. The nearly colourless oil solidified to a colourless solid melting in the neighbourhood of 40°. The solid was taken up in ether and glacial acetic acid added in an equivalent amount to form a diacetate. A white crystalline solid separated which was recrystallized from ethyl acetate: White, glistening flakes, m.p. 99° (bar). Calcd. for $C_{24}H_{36}N_2 \cdot 2CH_3COOH$: N, 5.94. Found: N, 5.87.

Example 21.

Preparation of 1:3-bis(benzylamino)-propane and salts thereof.

45 g. of benzylamine and 40.4 g. of trimethylene bromide were warmed on a steam-bath. In a few minutes a vigorous reaction set in, the liquid became very viscous and then rapidly solidified to a crystalline mass. The mixture was made alkaline with sodium hydroxide solution, extracted with ether, the ether layer then extracted with dilute acetic acid. The acid layer was made alkaline and extracted with ether. The ether layer was dried over magnesium sulphate, filtered through carbon, the ether removed and the residue fractionated. The 1:3 - bis - (benzylamino)-propane had b.p. 124° at 0.55 mm., n_D^{25} 1.5580.

The acetate was prepared by solution of the base in ether and addition of glacial acetic acid as long as a precipitate formed. The solid was filtered off, washed with ether and recrystallized from ethyl acetate. Small, white crystal-

line flakes, m.p. 132°. Calcd. for $C_{17}H_{22}N_2 \cdot 2CH_3COOH$: N, 7.49. Found: N, 7.29.

The hydrobromide was obtained by washing a portion of the original solid reaction product with water and recrystallizing first from water and then from alcohol. Small, white glistening flakes, m.p. 294°. Calcd. for $C_{17}H_{22}N_2 \cdot 2HBr$: N, 6.73; Br, 38.4. Found: N, 7.0; Br. 38.4. The picrate had m.p. 143° dec.

EXAMPLE 22.

PREPARATION OF 1:5-BIS-(BENZYLAMINO)-PENTANE AND A SALT THEREOF.

39.7 g. of pentamethylene iodide and 27 g. of benzylamine were brought together. Much heat was evolved and after cooling somewhat the reaction mixture was warmed an hour on the steam-bath. The clear syrupy material on standing for several days became crystalline. Water was then added and the mixture was made alkaline and extracted with ether. The ether layer was extracted with dilute acetic acid. The acid extract was made alkaline and extracted with ether. The ether layer was dried over magnesium sulphate, filtered through carbon and the ether removed. The residue on fractionation yielded unchanged benzylamine, N-benzyl piperidine and 1:5-bis-(benzylamino)-pentane. B.p. 121°/0.85 mm., $n_D^{22.5}$ 1.5577.

The acetate prepared by adding glacial acetic acid to an ethereal solution of the base, formed fine white crystals, m.p. 102°.

EXAMPLE 23.

PREPARATION OF N:N¹ - BIS - (BETA-PHENYLETHYL) - ETHYLENEDIAMINE AND A SALT THEREOF.

48.2 g. of beta-phenylethylamine and 18.8 g. ethylene bromide were warmed on the steam-bath. The reaction was vigorous and after standing some time at room temperature the mass crystallized. The reaction product was made alkaline, extracted with ether and the ether layer extracted with dilute acetic acid. The acid extract was made alkaline, extracted with ether; the ether layer dried over magnesium sulphate, filtered through carbon and the ether removed. The residue was then distilled; all volatile material was taken off up to 210° at 1.5 mm. The residue was taken up in ether and treated with glacial acetic acid. The solid was filtered off and recrystallized from ethyl acetate to give small white needles, m.p. 114°.

In the same manner as taught in Examples 8—10 and in the last three Examples 21—23, an alkylene dihalide such as 1:2-dichloroethane may be reacted with laurylamine, 3-methyl-cyclo-hexylamine, *m*-nitraniline, 2-amino-6-methyl-pyridine, 2-amino-4-methyl-pyridine, 2-amino-5-methyl-pyridine, 2-amino-3-methyl-pyridine, 2-amino-thiazole, or 2-amino-5-methyl-furan to form the symmetrically disubstituted alkylene diamine and desired salts thereof. The reaction mixture, after making strongly basic with alkali such as sodium hydroxide, may be extracted with ether and the ether extract distilled to obtain the desired product as a crystalline or solid residue.

EXAMPLE 24.

PREP. NO. 1 OF N:N¹-BIS-(*p*-HYDROXYBENZYL)-ETHYLENEDIAMINE AND ITS SALTS.

N-(*p*-hydroxybenzyl) - ethylenediamine dihydrochloride (7.2 g., 0.03 mol.), 3.7 g. (0.03 mol.) *p*-hydroxybenzaldehyde and 4 g. (0.06 mol.) sodium formate were mixed with 25 ml. 98—100% formic acid and heated under reflux for 20 hours with evolution of carbon dioxide. Hydrochloric acid (20 ml. 6N) was added to the reaction mixture, which was heated under reflux one hour, and then taken to dryness under reduced pressure. The solid residue was taken up in hot alcohol, filtered to remove sodium chloride, and acetone added to the hot filtrate to crystallize the product, N:N¹-bis-(*p*-hydroxybenzyl) - ethylenediamine dihydrochloride, m.p. 242° (bar). The dipicrate was recrystallized from aqueous alcohol, m.p. 193°.

EXAMPLE 25.

PREP. NO. 2 OF N:N¹-BIS-(*p*-HYDROXYBENZYL)-ETHYLENEDIAMINE AND SALTS THEREOF.

N:N¹ - bis - (*p*-methoxybenzyl) - ethylenediamine dihydrochloride (10 g.), 50 ml. 48% hydrobromic acid, and 15 ml. glacial acetic acid were heated under reflux for several hours. After cooling, insoluble starting material was removed by filtration, and the filtrate taken to dryness on the steam bath. The solid residue was taken up in hot isopropanol, filtered to remove some starting material, and the hot filtrate diluted with hexane to the cloud point.

On cooling, there crystallized N:N¹-bis-(*p*-hydroxybenzyl) - ethylenediamine dihydrobromide, m.p. 218° (bar). The dipicrate was recrystallized from aqueous alcohol, m.p. 193° (no depression of m.p. when mixed with picrate of preceding preparation).

EXAMPLE 26.

PREP. NO. 2 OF THE DIHYDROCHLORIDE OF N:N¹-DIPIPERONYLETHYLENEDIAMINE.

Piperonal (15 gm. 0.1 mol.) and ethylenediamine diformate (7.6 g. 0.05 mol.) were mixed with 100 ml. alcohol and heated under reflux for 48 hours. Concentrated hydrochloric acid (11 ml., 0.13 mol.) was added, the reaction mixture concentrated on the steam bath and cooled. White crystals formed which were removed by filtration and washed with alcohol. Yield 10.9 g. (54%) m.p. 264° (bar). Recrystallized from aqueous ethanol, m.p. 300° (bar).

EXAMPLE 27.

PREPARATION OF N:N-DIBENZYLETHYLENEDIAMINE.

(a) 2-(N:N-DIBENZYLAMINO)-ETHANOL.

2-Aminoethanol (122 gms. 2 mols) was stirred and warmed to about 40° C. and 257 gm. (2 mols) of benzyl chloride was added dropwise. With continued stirring, the reaction mixture was heated to 100—110° C. for 3 hours. On cooling 80 gms. of sodium hydroxide in 120 ml. of water was added and the reaction

mixture was stirred at 100° C. for one hour. It was then extracted with benzene, the benzene solution washed with water, dried and concentrated. The residue was then distilled under reduced pressure.

Fraction	B range	mm.	n_D^{25}	Wt. gms.
I	110—118° C.	0.2	1.5198	114
II	155—170° C.	0.2	1.5615	56.3

Fraction II was the desired product and was identified by conversion to the hydrochloride, m.p. 183—4° C.

(b) 2-(N:N-DIBENZYLAMINO) ETHYL CHLORIDE HYDROCHLORIDE.

Fifty grams of dibenzylaminoethanol dissolved in 50 ml. chloroform was cooled in an ice bath, and while being stirred 28 gms. of thionyl chloride was added slowly. The mixture was stirred three hours at 15—25° C. then allowed to stand at room temperature. The solvent was taken off under vacuum and the residue recrystallized from ethanol: m.p. 192—3° C.

(c) N:N-DIBENZYLETHYLENEDIAMINE.

Fourteen gms. of β-N:N-dibenzylaminoethyl chloride hydrochloride was dissolved in 75 cc. of anhydrous ammoniacal ethanol (saturated at 20° C.) and sealed into a bomb-tube. The tube was heated at 85—90° C. for 6 hours. On cooling a precipitate of ammonium chloride was obtained and filtered off. The filtrate was concentrated under vacuum. The solid residue was washed with ether and dried, m.p. 145—9° C. This was stirred with 20% aqueous sodium hydroxide, the mixture extracted with benzene and the extracts dried. The benzene was removed under vacuum and the residue distilled.

B.P. 175—185° C./1 mm., n_D^{24} 1.5682. Yield 5.5 gm. (45%). The dipicrate had m.p. 209—210° C.

Following the procedure disclosed hereinabove the following compounds may be made.

Monosubstituted diamines	Starting materials
N-2-thenylethylenediamine	thiophene, formaldehyde, ethylenediamine-di-HCl salt
N-*p*-chloro or bromophenylethylene diamine	N-phenylethylene diamine and Cl_2 or Br_2
N-2:4 or other dichloro or bromophenylethylene diamine	N-phenylethylene diamine and Cl_2 or Br_2
N-2- or 4-pyridylethylethylenediamine	2- or 4-picoline, formaldehyde, ethylenediamine di-HCl salt
N-2- or 4-quinolylethylethylethylenediamine	2- or 4-quinaldine, formaldehyde, ethylenediamine di-HCl salt
N-4-methoxycyclohexylethylenediamine	4-methoxycyclohexanone and ethylenediamine diformate
Disubstituted diamines	
N-cyclohexyl-N'-ethylethylene diamine	N-cyclohexylethylene diamine, acetaldehyde, H_2
N-benzyl-N'-vanillylethylene diamine	N-benzylethylene diamine, vanillin, formic acid
N-methyl-N-phenylethylene diamine	methyl aniline, chloracetal, followed by ammonium formateformic acid

Any of the above N-substituted diamines as well as any of the N-substituted diamines disclosed in Application No. 28136/53 (Serial No. 716,598) or the salts of any such N-substituted diamines, may be employed for the preparation of penicillin salts by the process of the present invention.

The following examples illustrate the preparation of penicillin salts from the corresponding substituted alkylene diamines.

EXAMPLE 28.

PREPARATION OF N-MONO-BENZYLETHYLENEDIAMINE DI-PENICILLIN.

224 mg. of N-mono-benzylethylenediamine dihydrochloride was dissolved in about 2 cc. of water. To this a solution of 712 mg. of sodium penicillin dissolved in 2 to 3 cc. of water was added. A clear solution resulted from which an oil precipitated in a few minutes. The water layer was poured off and the oil placed in a desiccator over phosphorus pentoxide. In a short time white crystals appeared and all of the oil solidified. On heating at any temperature between 124 and 191°, this material melted and immediately solidified to a white foamy, very viscous mass. At 191° (bar) the solid melted to a liquid which darkened and decomposed without giving the white foamy mass.

Example 29.

PREPARATION OF N-MONO-(*p*-HYDROXYBENZYL)-ETHYLENEDIAMINE DIPENICILLIN.

240 mg. of N-mono-(*p*-hydroxybenzyl)-ethylenediamine dihydrochloride was dissolved in about 2 cc. of water. To this solution 712 mg. of sodium penicillin dissolved in about 2 to 3 cc. of water was added. An immediate white granular precipitate formed which was filtered off, washed with water and dried over phosphorus pentoxide. The m.p. was 131° (Bar).

Example 30.

N:N-DIBENZYLETHYLENEDIAMINE MONOPENICILLIN.

Half a gram of the base was dissolved in 1 ml. water containing 0.125 ml. acetic acid. This solution was added to a solution of 0.78 gms. potassium penicillin in 1 ml. water. A syrup rapidly formed. The supernatant aqueous layer was removed and the syrup washed with water. The syrup was then transferred to a Petri dish and dried in a vacuum desiccator over phosphorus pentoxide. After drying it was powdered and then showed a melting range of 50—55° C. (indefinite) to a viscous mass.

Example 31.

PREPARATION OF N:N¹-DIBENZYLETHYLENEDIAMINE DI-PENICILLIN.

To a solution of 60 g. of sodium penicillin G in 800 cc. of distilled water cooled to 0—4° in an ice-bath, a solution of 35 g. of N:N¹-dibenzylethylenediamine diacetate in 200 cc. of distilled water is added dropwise with stirring. The thick slurry is filtered with suction, washed twice with 100 cc. of cold water, dried by suction and spread out in a thin layer for completion of drying. The product weighed 80 g.

The air-dried powder has a broad melting point, sintering at 100°, melting above 110° to a cloudy liquid becoming clear at 135°. It has 7.7—9.6% water (of crystallization), which can be removed on drying in a Fischer pistol. Analysis of the dried sample showed: C, 63.86; H, 6.41; S, 6.74. Calcd. for $2C_{16}H_{18}O_4N_2S \cdot C_{16}H_{20}N_2$: C, 63.8; H, 6.20; S, 7.08. Ash was negligible. Assay showed on a dry basis 1235 units per mg. (calcd. 1307).

Example 32.

PREPARATION OF N:N¹-DIPIPERONYLETHYLENEDIAMINE DI-PENICILLIN.

To a solution of 1.07 g. sodium penicillin in about 2 ml. cold water, a solution of 0.7 g. N:N¹-dipiperonylethylene-diamine diacetate in about 2 ml. cold water was slowly added with stirring. The white insoluble solid was filtered off, washed with water and dried over phosphorus pentoxide.

Example 33.

PREPARATION OF N:N¹-DIFURFURYLETHYLENEDIAMINE DI-PENICILLIN.

Sodium penicillin (1.07 g.) is dissolved in about 2 ml. water cooled in an ice-bath. To the clear solution, 0.5 g. of N:N¹-difurfuryl-ethylenediamine diacetate dissolved in about 2 ml. of cold water is slowly added with stirring. A white, insoluble, crystalline powder is formed which is filtered off, washed with cold water and dried in a desiccator over phosphorus pentoxide.

The insoluble organic base penicillin salts formed contain two mols. of penicillin for each mol. of organic base when a di-salt of the substituted diamine is used and when an excess of penicillin salt is present, as is indicated by the analysis of the N:N¹-dibenzylethylenediamine salt and by the assays of the N:N¹-dibenzyl, N:N¹-difurfuryl and N:N¹-dipiperonylethylenediamine penicillin salts obtained by Examples 31 to 33.

Besides the penicillin salts hereinabove mentioned, the following mono- and di-penicillin salts are also deemed useful for the reasons indicated.

N-octylethylenediamine di-penicillin (m.p. 98° C.).

N:N¹-di-*n*-(*iso*, *sec.* or *tert.*) butylethylene diamine di-penicillin. (The *n*-butyl compound has a potency of 1170 units per mg., the iso-butyl compound has a potency of 1415 units per mg. and a m.p. of 127—129° C. and the *sec*-butyl compound has a potency of 1270 units per mg.).

N:N¹-di-phenylethylenediamine di-penicillin (m.p. 110—130° C. and a potency of 1335 units per mg.).

N:N¹-di-1 (or 2)-naphthyl ethylene diamine di-penicillin.

N:N¹-di-2-pyridylethylenediamine di-penicillin.

N-benzhydrylethylenediamine di-penicillin.

N:N¹-di-benzhydrylethylenediamine di-penicillin (m.p. 118—119° C. and a potency of 1012 units per mg.).

N-veratrylethylenediamine di-penicillin.

N:N¹-di-veratrylethylenediamine di-penicillin.

N-benzyl-N¹-*p*-methoxybenzylethylenediamine di-penicillin.

N:N¹-dibenzyl-1-methylethylenediamine di-penicillin (m.p. 133° C. with decomposition and a potency of 1130 units per mg.).

N:N-dicyclohexylethylenediamine mono-penicillin.

N:N-diamyl ethylenediamine mono-penicillin.

N-ethyl-N-phenylethylenediamine mono-penicillin.

The penicillin salts corresponding to the illustrative examples are listed with their melting points:

Organic base salt	Form of di-penicillin salt	M.P. °C.
N:N¹-dibenzylethylenediamine diacetate	White crystalline powder	ca. 110—135
N:N¹-dipiperonylethylenediamine diacetate.	White powder or colourless crystals	112—119
N:N¹-bis-(*p*-chlorobenzyl)-ethylenediamine diacetate	White crystals	110—117
N:N¹-bis-(2:4-dichlorobenzyl)-ethylenediamine diacetate	Yellowish prisms	95—100
N:N¹-bis-(*p*-nitrobenzyl)-ethylenediamine diacetate	White crystals	95—100
N:N¹-bis-(*p*-hydroxybenzyl)-ethylenediamine dihydrochloride	White crystals	140—145
N:N¹-bis-(*p*-methoxybenzyl)-ethylenediamine diacetate	White crystals	100—103
N:N¹-bis-(*p*-aminobenzyl)-ethylenediamine dihydrochloride	White, granular crystals	144—8
N-benzyl-N¹-(alpha-ethylbenzyl)-ethylenediamine dihydrochloride	White crystals	105—8
N:N¹-bis-(beta-phenylethyl)-ethylenediamine diacetate	White crystals	95—102
N:N¹-bis-(gamma-phenylpropyl)-ethylenediamine dihydrochloride	White crystals	78—83
1:3-bis-(benzylamino)-propane diacetate	White crystals	100—102
1:5-bis-(benzylamino)-pentane diacetate	White glass	120—140
N:N¹-difurfurylethylenediamine diacetate	White crystalline powder	83—86
N:N¹-di-2-thenylethylenediamine diacetate	White crystals	145—7
N:N¹-dicyclohexylethylenediamine diacetate	White crystals	155—6
N:N¹-bis-(4-methyl-2-pentyl)-ethylenediamine diacetate	Colourless solid	165
N:N¹-bis-(2-heptyl)-ethylenediamine diacetate	Crystalline solid	75—85
N:N¹-bis-(3:5:5-trimethylhexyl)-ethylenediamine diacetate	White crystals	90—95
N:N¹-bis-(*p*-methylbenzyl)-ethylenediamine diacetate	White crystals	90—97
1:10-bis-(benzylamino)-decane diacetate	White solid	106

These salts are insoluble in most of the usual laboratory solvents, but have very appreciable solubility in dimethyl formamide. By precipitating the salts from this solvent by addition of water judiciously, the particle size can be adjusted to any desired magnitude. Recrystallization may also be effected from acetone and formamide.

The solubility properties of the penicillin salts of the invention are such that many substituted alkylene diamines could be used in any stage of the recovery process for the isolation of penicillin. Since a substantial number of compounds, particularly those of higher molecular weight, are either insoluble or only sparingly soluble in water, a precipitation from the buffered penicillin extract is possible. With regard to N:N¹-dibenzylethylenediamine, for example, the free base could be used for precipitation of penicillin from the amyl acetate extracting liquors since the base is soluble in this medium. The diacetate or any other water-soluble salt could, of course, be used for the aqueous precipitation. The versatility of these compounds is clearly obvious.

When the diamine penicillin salts are used for therapeutic purposes, they may be used for injection either with a spreading agent such as hyaluronidase, with a suspending agent such as carboxymethyl cellulose or pectin, or in a vegetable oil vehicle either with beeswax or aluminium mono-stearate gel, the composition

being made up in substantially the same manner as any other sparingly soluble penicillin salt known to the art.

The diamine penicillin salts herein specifically disclosed vary in their solubility in water from sparing solubility to substantial insolubility. Because of this action, there is a variation in their therapeutic effects ranging from quickly measurable blood levels to delayed blood level measurements. The clinician is therefore given a wide choice of penicillin products with varying times of onset of action. Further variations may be made by mixing fast-acting and slow-acting compounds, whether this involves combinations of different diamine penicillins or combinations of procaine penicillin or other known compounds with one or more compounds of the invention.

What we claim is:—

1. New salts of penicillin and N-substituted diamines, said N-substituted diamines having the general formula

$$\begin{matrix} R \\ \\ R_1 \end{matrix} \!\!>\!N\!-\!Y\!-\!NHR_2$$

wherein

R represents an aliphatic, alicyclic, araliphatic, aromatic or heterocyclic group which may or may not have substituents,

R_1 represents a hydrogen atom or an aliphatic, alicyclic, araliphatic, aromatic or heterocyclic group which may or may not have substituents,

R_2 represents a hydrogen atom or, when R_1 is hydrogen, an aliphatic, alicyclic, araliphatic, aromatic or heterocyclic group which may or may not have substituents, and

Y represents an alkylene radical having from 2 to 12 carbon atoms.

2. The new compound N:N'-dibenzyl-ethylene diamine di-penicillin.

3. The new compound N:N'-dicyclohexyl-ethylene diamine di-penicillin.

4. The new compound N:N'-bis-(*p*-chlorobenzyl)-ethylene diamine di-penicillin.

5. The new compound N:N'-dipiperonyl-ethylene diamine di-penicillin.

6. The new compound N:N'-difurfuryl-ethylene diamine di-penicillin.

7. The new compound N:N'-di-isobutyl-ethylene diamine di-penicillin.

8. The new compound N:N'-bis-(3:5:5-trimethyl-hexyl)-ethylene diamine di-penicillin.

9. Salts of penicillin and N-substituted diamines as claimed in any of claims 1 to 8 in which the N-substituted diamine has been obtained by reacting an alkylene diamine with a ketone or aldehyde and hydrogenating the resulting substituted di-imine.

10. Salts of penicillin and N-substituted diamines as claimed in any of claims 1 to 8 in which the N-substituted diamine has been obtained by reacting an alkylene dihalide with an amine.

11. Salts of penicillin and N-substituted diamines as claimed in any of claims 1 to 8 in which the N-substituted diamine has been obtained by reacting a salt of ethylene diamine with formaldehyde and a compound containing an active hydrogen atom.

12. Salts of penicillin and N-substituted diamines as claimed in any of claims 1 to 8 in which the N-substituted diamine has been obtained by reacting ethylene diamine diformate with an aldehyde or ketone in a solvent.

13. The process of making a salt of penicillin which comprises bringing penicillin as such into contact with an N-substituted diamine or alternatively, bringing a salt of penicillin into contact with a salt of an N-substituted diamine, said N-substituted diamine in either case having the general formula given in claim 1.

14. The process as claimed in claim 13 in which an aqueous solution of a salt of penicillin is mixed with an aqueous solution of a salt of an N-substituted diamine having the general formula given in claim 1 and thereafter the salt of penicillin with said N-substituted diamine is separated from the aqueous reaction mixture.

15. The process as claimed in claim 14 in which an aqueous solution of an alkali metal salt of penicillin is used.

16. The process as claimed in claim 14 or 15 in which an aqueous solution of a di-salt of the N-substituted diamine is used.

17. The process as claimed in claim 16 in which there is used an aqueous solution of a di-salt of the N-substituted diamine with such an organic acid that the di-salt has a solubility exceeding 5 gms. per 100 cc. of solution at 30° C.

18. The process as claimed in claim 16 in which an aqueous solution of the diacetate of the N-substituted diamine is used.

19. The process as claimed in any of claims 13 to 18 in which the N-substituted diamine is N:N'-dibenzyl-ethylene diamine.

20. The process of making a substantially water-insoluble diamine salt of penicillin comprising reacting an aliphatic, araliphatic, aromatic or heterocyclic halide with an alkylene diamine thus forming an N:N'-disubstituted alkylene diamine in the form of its halide salt, treating the latter with an alkali and extracting the substituted alkylene diamine in the form of a free base, reacting the free base with an aliphatic carboxylic acid or acid anhydride to form the carboxylic acid salt of the substituted alkylene diamine and finally reacting the latter with a penicillin salt in an aqueous medium to form the substantially water-insoluble diamine salt of penicillin.

21. The processes of making salts of penicillin particularly described in any of Examples 28 to 31.

W. P. THOMPSON & CO.,
12, Church Street, Liverpool, 1,
Chartered Patent Agents.

CORRECTION OF CLERICAL ERROR

SPECIFICATION NO. 716,556

The following correction is in accordance with the Decision of the Superintending Examiner, acting for the Comptroller-General, dated the fifteenth day of December, 1954.

Page 14, line 3, *delete* "27 to 31" *insert* "28 to 33".

THE PATENT OFFICE,
19th January, 1955

DB 72566/1(9)/3613 150 1/55 R

PUBLISHED BY :-
THE PATENT OFFICE,
25, SOUTHAMPTON BUILDINGS,
LONDON, W.C.2.

"Ribbon accompanying seal or fastener was cut or removed during data conversion for the purposes of scanning in the document. Date 15 JUN 2000

W. P. Thompson