refluxed for 3 hours. During this period the starting material dissolved. The reaction mixture was cooled, filtered to remove a small amount of suspended solid and extracted several times with chloroform. Removal of the chloroform by evaporation left a light-tan oil which crystallized on standing. The compound was recrystallized (with some difficulty) from benzene, giving 1.2 g. (83% yield) of white needles, m.p. 99–100°. Crystallization from commercial heptanes is less difficult.

Anal. Calcd. for $C_{11}H_{11}O_2N$: C, 69.82; H, 5.86; N, 7.40. Found: C, 70.10; H, 5.95; N, 7.63.

The compound was obtained in 69% yield by a similar hydrolysis of the corresponding methyl ester, 4-carbomethoxy-1-benzyl-2,3-dioxopyrrolidine (IX).

Preparation of β-Benzylaminopropionic Acid.—A mixture of 30 g. of ethyl β-benzylaminopropionate[12] and 150 ml. of 10% sodium hydroxide solution was refluxed until the ester layer disappeared. The solution was neutralized with dilute hydrochloric acid and evaporated to dryness. The resulting white solid was extracted with boiling absolute ethanol, and the alcohol extract was cooled to give 22.5 g. (87%) of white needles of β-benzylaminopropionic acid, m.p. 182–183°. The reported[16] m.p. is 181°.

Preparation of N-Methoxalyl-β-benzylaminopropionic Acid.—A mixture of 5 g. of β-benzylaminopropionic acid and 10 g. of methoxalyl chloride was heated on the steam-bath for 30 minutes. The solution was diluted with 100 ml. of ether and washed with water, then extracted with an excess of 5% sodium bicarbonate solution. Acidification of the sodium bicarbonate solution precipitated a light yellow oil, which crystallized on standing. This solid was recrystallized from chloroform–petroleum ether (or ether–petroleum ether) and gave 5 g. (68%) of white plates of N-methoxalyl-β-benzylaminopropionic acid, m.p. 99–100°.

Anal. Calcd. for $C_{13}H_{15}O_5N$: C, 58.86; H, 5.70; N, 5.28. Found: C, 58.86; H, 5.52; N, 5.37.

Preparation of 1-Benzyl-2-oxo-3-methoxy-3-pyrroline-4-carboxylic Acid (XI).—To a solution of 8 g. of N-methoxalyl-β-benzylaminopropionic acid and 8 g. of methoxalyl chloride in 25 ml. of anhydrous chloroform was added 8 g. of anhydrous pyridine. The solution was allowed to stand for 2 hours and it assumed a deep red color. The chloroform solution was washed with water, then heated on the steam-bath with 400 ml. of 5% sodium bicarbonate solution. The bicarbonate extract was decolorized with charcoal and filtered. Acidification of the solution precipitated 2.2 g. of an unidentified acid, which was removed by filtration. Chloroform extraction of the filtrate yielded 0.5 g. of 1-benzyl-2-oxo-3-methoxy-3-pyrroline-4-carboxylic acid (XI), obtained as very small white needles, m.p. 139–140°, after crystallization from chloroform–petroleum ether.

Anal. Calcd. for $C_{13}H_{13}O_4N$: C, 63.15; H, 5.30; N, 5.66. Found: C, 62.77, 63.03; H, 5.02, 4.97; N, 5.64.

Esterification of this compound with diazomethane yielded 4-carbomethoxy-1-benzyl-2-oxo-3-methoxy-3-pyrroline (X), m.p. 77–78°, identical with the product obtained by the treatment of 4-carbomethoxy-1-benzyl-2,3-dioxopyrrolidine (IX) with diazomethane.

The unidentified acid mentioned above was purified by solution in aqueous potassium hydroxide and reprecipitation with hydrochloric acid, followed by recrystallization from aqueous ethanol. Very small pale yellow plates, m.p. 245–246°, were obtained.

Anal. Found: C, 55.32, 55.17; H, 3.53, 3.27; N, 5.85.

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(16) J. A. King and F. H. McMillan, THIS JOURNAL, **68**, 1468 (1946).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

Stereochemistry of the β-Phenylserines: Characterization of Allophenylserine[1]

BY KENNETH N. F. SHAW[2] AND SIDNEY W. FOX

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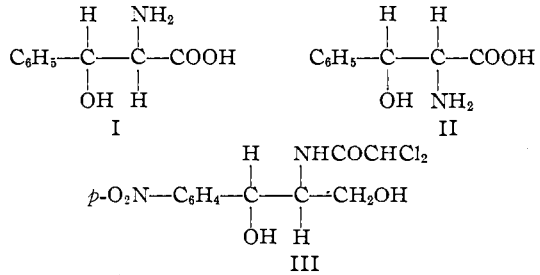
Phenylserine was converted *via* lithium aluminum hydride reduction of its esters to racemic chloramphenicol. *Threo* configuration of the hydroxyamino acid was thus indicated. In addition to phenylserine, the disputed allophenylserine was obtained from condensation of benzaldehyde with glycine. This diastereomer was separated from the crude reaction product as the ethyl ester hydrochloride. Its structure and configuration were established by conversion of the ethyl ester to *erythro* intermediates in the chloramphenicol series.

Although phenylserine (I) has been well known for six decades, its diastereomer, allophenylserine (II), has been a subject of contention, partly due to its confusion with one of the two α-hydroxy-β-amino acids, the β-phenylisoserines. Interest in clarifying the stereochemistry of the phenylserines and their analogs arises from their structural similarity and possible biochemical relation to natural products, such as epinephrine, chloramphenicol (III) and the α-amino acids.



I

II

III

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(2) From the Ph.D. dissertation of Kenneth N. F. Shaw, 1951.

Synthesis of III from I, which was the initial aim of the present study, had been achieved[3] when reports of similar but earlier investigations[4,5] became available. Subsequent publications[6–11] have reflected the parallel interest of other workers in this problem. In view of such activity, attention in this Laboratory was turned to phenylserine synthesis, with emphasis on the disputed II. It may be noted that discrepancies between different reports have been attributed to possible contamination of I by II.[7]

I was first prepared by Erlenmeyer[12,13] by con-

(3) K. N. F. Shaw and S. W. Fox, Abstracts of Papers, 118th Am. Chem. Soc. Meeting, p. 28N (1950).

(4) C. G. Alberti, B. Asero, B. Camerino, R. Sannicolò and A. Vercellone, *Chimica e industria* (Milan), **31**, 357 (1949).

(5) G. Carrara and G. Weitnauer, *Gazz. chim. ital.*, **79**, 856 (1949).

(6) K. Vogler, *Helv. Chim. Acta*, **33**, 2111 (1950).

(7) C. F. Huebner and C. R. Scholz, THIS JOURNAL, **73**, 2089 (1951).

(8) K. Hayes and G. Gever, *J. Org. Chem.*, **16**, 269 (1951).

(9) G. W. Moersch (to Parke, Davis and Co.), U. S. Patent 2,538,792 (Jan. 23, 1951).

(10) E. D. Bergmann, H. Bendas and W. Taub, *J. Chem. Soc.*, 2673 (1951).

(11) H. E. Carter and E. H. Flynn (to Eli Lilly and Co.), U. S. Patent 2,556,868 (June 12, 1951).

(12) E. Erlenmeyer, Jr., *Ber.*, **25**, 3445 (1892).

(13) E. Erlenmeyer, Jr., and E. Früstück, *Ann.*, **284**, 36 (1894).

densation of glycine with benzaldehyde, this procedure being improved subsequently.[14,15] Monohydrated I was reported to melt with decomposition at 192–193°[12] and 193–194°[13] and the anhydrous compound at 195–196°,[12] 190°[13] and 200–202°.[14]

$$2C_6H_5CHO + NH_2CH_2COOH \xrightarrow[EtOH\ H_2O]{NaOH} C_6H_5CHOHCHCOONa \xrightarrow{HOAc} I$$

(with $C_6H_5\cdot CH{=}N$ on the CH)

In the present study, I was prepared according to the German patent procedure.[15] Condensation was effected in an entirely aqueous medium with a minimal quantity of sodium hydroxide. The N-benzal intermediate was hydrolyzed with hydrochloric acid without isolation. This procedure was found more satisfactory than others which were tested with respect to yield, ease of processing and freedom from side reactions. Crude I was recrystallized to give fractions which decomposed in the range 189–197°, variation occurring with initial bath temperature and rate of heating. Degree of hydration, procedural modification and number of the crystal crop had no significant effect. Thus, decomposition temperature gave no indication that more than the single diastereomer was present.

En route to III, twice recrystallized I[16] was treated with hydrogen chloride in the appropriate alcohol to obtain the methyl and ethyl ester hydrochlorides. The latter compound has been briefly mentioned in earler literature.[17–19] The ester hydrochlorides are not hygroscopic and can be stored indefinitely at room temperature.

The free esters were prepared in almost quantitative yield by briefly bubbling ammonia gas through ether suspensions of the ester hydrochlorides. A 2% solution of ammonia in cold chloroform, which has been used for synthesis of other amino acid esters,[20] proved unsatisfactory, due to formation of ammonium chloride in a troublesome colloidal state. The recrystallized esters can be stored in absence of moisture for several weeks, although melting points of declining value suggest slow deterioration. Impure products, or those open to the atmosphere, degenerate more rapidly to oily gums smelling of benzaldehyde and ammonia.

The esters of I were reduced with lithium aluminum hydride to phenylserinol (DL-*threo*-1-phenyl-2-amino-1,3-propanediol), a key intermediate in the original chloramphenicol synthesis.[21] The vigorous reduction reaction was conveniently controlled by introducing the ester *via* Soxhlet extraction. Reasonable recovery of the product was achieved by twelve ether extractions of the quenched reaction mixture. Soxhlet extraction with ether at this point was less satisfactory (*cf.* allophenylserinol, under Experimental), due to poor permeability of aluminum hydroxide residues. Recovery was lower when ethanol or chloroform were used for extraction.

The compounds listed in Table I were prepared by known procedures.[21]

TABLE I

CHLORAMPHENICOL INTERMEDIATES

Compound	M.p., °C. Lit.[21]	Found
N-Acetylphenylserinol	136–17	134–135
N,O-Diacetylphenylserinol	168–169	168–169
Triacetylphenylserinol	79–80	81–82
p-Nitrophenylserinol	141.5–142.5	140–141
N-Dichloroacetyl-*p*-nitrophenylserinol (racemic chloramphenicol)	150.5–151.5	150–151[a]

[a] Unchanged by mixture with an authentic sample kindly supplied by Eli Lilly and Co.

The final product was approximately one-half as active as the natural antibiotic in microbiological assay.[22]

The *threo* configuration of I and its consequent similarity to the natural amino acid threonine, indicated by the transformation to III, has been confirmed by use of other chemical sequences,[6,23] and by enzymic degradation.[24] This feature, as well as the availability of I, is of advantage in synthesis of III, since troublesome separation of isomers at the aminediol state[21] is obviated.

Erlenmeyer mentioned having obtained small amounts of II, m.p. 187–188°, from the mother liquors of glycine–benzaldehyde condensation.[25] II was also noted as a minor by-product of phenylisoserine synthesis, in which ammonia was added to one of the sodium phenylglycidate isomers.[13,26] Erlenmeyer's claims concerning II were disregarded or questioned by subsequent investigators.[14,27] Forster and Rao,[14] who assigned a *trans* configuration to I, described the preparation of a *cis*-diastereomer, which was subsequently shown to be one of the phenylisoserines.[28] An "*erythro*-β-phenylserine," m.p. 260°, has been recently reported, from catalytic hydrogenation of ethyl α-oximinobenzoylacetate,[29] but only sparse confirmatory data were provided.

In this Laboratory, the recrystallization filtrates and alcoholic washings from crude I were processed to give further crops, which showed the same decomposition range but much finer crystal appearance than pure phenylserine. When these materials were treated with hydrogen chloride in

(14) M. O. Forster and K. A. N. Rao, *J. Chem. Soc.*, 1943 (1926).

(15) Ges. für Kohlentechnik m.b.H., German Patent 632,424 (July 8, 1936).

(16) A method for evaluating steric purity is presented in a following paper, K. N. F. Shaw and S. W. Fox, THIS JOURNAL, **75**, 3421 (1953).

(17) E. Abderhalden and S. Buadze, *Fermentforschung*, **8**, 487 (1926).

(18) F. Bettzieche and R. Menger, *Z. physiol. Chem.*, **172**, 64 (1927).

(19) D. G. Doherty, J. E. Tietzman and M. Bergmann, *J. Biol. Chem.*, **147**, 617 (1943).

(20) G. Hillmann, *Z. Naturforsch.*, **1**, 682 (1946).

(21) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., THIS JOURNAL, **71**, 2463 (1949).

(22) The authors are grateful to workers in the Upjohn Co. for carrying out the microbiological assay.

(23) D. Billet, *Compt. rend.*, **230**, 1074 (1950).

(24) W. S. Fones, *Arch. Biochem. Biophys.*, **36**, 486 (1952).

(25) E. Erlenmeyer, Jr., *Ann.*, **307**, 70 (1899).

(26) E. Erlenmeyer, Jr., and C. Barkow, *Ber.*, **39**, 791 (1906).

(27) E. Fourneau and J. R. Billeter, *Bull. soc. chim.*, [5] **7**, 593 (1940).

(28) M. Oesterlin, *Metallbörse*, **19**, 1237 (1929).

(29) I. Elphimoff-Felkin and H. Felkin, *Compt. rend.*, **232**, 241 (1951).

ethanol, there precipitated an ethyl ester hydrochloride different in physical properties from that of I. The new compound was converted to its parent ester by treatment with ammonia in ether. Analytical data suggested that the two substances were isomers of the corresponding derivatives of I. This was confirmed by lithium aluminum hydride reduction of the ester to DL-*erythro*-1-phenyl-2-amino-1,3-propanediol (allophenylserinol). For further characterization, the known compounds[21] listed in Table II were prepared.

TABLE II

ALLOPHENYLSERINOL DERIVATIVES

Compound	M.p., °C. Lit.[21]	Found
N-Acetyl	106.5–107	108
N,O-Diacetyl	110–111	109–110
Triacetyl	115–116	116

Free II,[16] named allophenylserine because of its configurational similarity to allothreonine, was obtained in good yield by alkaline hydrolysis of its ethyl ester hydrochloride at room temperature. II and I decompose in the same temperature range, alone or mixed, but are individually distinct in crystal form. The identity of II has been confirmed by Fones,[24] who recently reported its degradation to L-mandelic acid by action of an L-amino acid oxidase preparation.

The proportion of II in crude condensation products and fractions resulting from recrystallization was determined by an indirect method. An aliquot was treated with hydrogen chloride in sufficient ethanol to dissolve the resulting I ethyl ester hydrochloride. The less soluble II ethyl ester hydrochloride which precipitated was weighed and the quantity remaining in solution was estimated from rough solubility data. With this method, it was found that recrystallization from five volumes of water lowered the II content of a mixture from an initial value of *ca.* 30% to *ca.* 10% (65% recovery). Alternatively, successive recrystallization of the same material from ten volumes of 50% methanol, then ten volumes of 50% ethanol gave pure I (42% recovery). Subsequent concentration of the recrystallization filtrates and treatment with dioxane led to optimum recovery of II-enriched fractions, from which II was obtained in pure form *via* its ethyl ester hydrochloride in the manner described earlier.

A mixture of ethyl ester hydrochlorides was obtained by concentration of the ethanol filtrates from which the allo compound had been removed.

TABLE III

SOLUBILITIES OF DIASTEREOMERIC ETHYL ESTER HYDROCHLORIDES[a]

Solvent	Temp., °C.	Ethyl ester hydrochloride, g./100 ml. Phenylserine	Allophenylserine
Ethanol	80	>100	5.0
	5	> 13	1.2–1.5
Acetone	55	22	0.06
	5	7.5	.04
Dioxane	95	160	.15
	14	4.7	.05

[a] Method is described in Experimental.

The solubilities of the individual ethyl ester hydrochlorides were then compared in various solvents, of which only acetone and dioxane showed promise for further fractionation (Table III).

Although the allo compound alone was poorly soluble in dioxane, it dissolved readily in presence of its diastereomer. The same effect was observed in acetone to a much smaller degree, so that recrystallization of the mixed ester hydrochlorides from this solvent permitted recovery of further small amounts of allo derivative.

Although both I and II were obtained in pure form, their recovery was incomplete. Lack of a simple method for distinguishing them impeded not only development of a more efficient mode of separation, but also investigation of the factors which controlled the ratio of isomers produced in the glycine–benzaldehyde condensation. Further study of these problems will be discussed in a following paper.[16]

Experimental[30]

Phenylserine (I).—The German patent procedure,[15] with some extensions, was employed. To a mechanically stirred solution of 30.0 g. (0.40 mole) of glycine and 24.0 g. (0.60 mole) of sodium hydroxide in 100 ml. of water at 15° was added 84.9 g. (0.80 mole) of benzaldehyde, with water-bath cooling at 15°. After standing overnight at room temperature, the resulting solid condensation cake was fragmented, and, with steady agitation and external cooling at 15°, 49 ml. (0.60 mole) of concentrated hydrochloric acid was slowly added. Following overnight storage at 5°, the crude I was filtered, and washed with three 250-ml. portions of boiling 95% ethanol. The white microcrystals thus obtained were dried over Anhydrone at 50–60° and 10–15 mm. to a constant weight of 50.5 g. (70% yield).

The ethanol-washed product was recrystallized from 1000 ml. of hot 50% aqueous methanol (45.3 g., 90% recovery) and then from 900 ml. of hot 50% aqueous ethanol to give 42.8 g. (85% recovery from the original 50.5 g.) of anhydrous I (shimmering hexagonal plates of I monohydrate before dehydration). The combined alcoholic filtrates were concentrated under reduced pressure to a moist residue: this was taken up in 80 ml. of boiling water, treated with the same volume of hot dioxane, and refrigerated overnight to give 9.0 g. (18% recovery) of white microcrystals (I + II).

The combined ethanol washings from the crude I were concentrated under reduced pressure to 100 ml. Following treatment with 100 ml. of ether, 11.2 g. of white powder was recovered, which showed a strongly positive ninhydrin test. This material, upon recrystallization from hot 66% aqueous dioxane, yielded a further 2.3 g. (5% yield) of mixed I and II.

Phenylserine Methyl Ester Hydrochloride.—A vigorous stream of dry hydrogen chloride was passed through a suspension of 9.0 g. (0.045 mole) of I monohydrate in 90 ml. of absolute methanol for two hours. Solution was accompanied by evolution of sufficient heat to promote gentle refluxing. No crystallization occurred upon overnight storage at −15°. The colorless solution was concentrated under reduced pressure to a crystalline residue which was dissolved in 50 ml. of warm methanol. Upon addition of 300 ml. of ether, two layers formed with rapid crystallization ensuing at the interface. Overnight storage at −15°, filtration, washing with ether and drying at 50–60° gave 8.6 g. (81% yield) of I methyl ester hydrochloride as white iridescent flakes, m.p. 160° (dec.), unchanged by recrystallization from methanol–ether (other values reported are 156°,[5] 161°[10] and 161–162°[11]).

Anal. Calcd. for $C_{10}H_{14}O_3NCl$: N, 6.04; Cl, 15.30. Found: N,[31] 6.11, 6.08; Cl (gravimetric), 15.31, 15.38, 15.33.

Phenylserine Ethyl Ester Hydrochloride.—This synthesis was conducted with absolute ethanol as described for the

(30) All melting points are uncorrected and were taken in open capillary tubes.

(31) N analyses by Mr. J. A. McMillan by micro Kjeldahl.

methyl homolog. An 85% yield was obtained, m.p. 140°, unchanged by recrystallization from ethanol–ether (other values reported are 134–135°,[32] 136–137°,[18] 137–139°,[19] 138°[5] and 164–165°[10]).

Anal. Calcd. for $C_{11}H_{16}O_3NCl$: N, 5.70; Cl, 14.43. Found: N, 5.61, 5.70; Cl (gravimetric), 14.32, 14.32, 14.42.

Phenylserine Methyl Ester.—A vigorous stream of dry ammonia was passed through a suspension of 11.5 g. (0.050 mole) of I methyl ester hydrochloride in 200 ml. of ether for 15 minutes. The granular ammonium chloride was filtered and washed with several portions of warm ether. Evaporation of the filtrate and washings gave 9.4 g. (97% yield) of crude I methyl ester, m.p. 60–61°. Recrystallization from hot 1:4 ether–cyclohexane yielded long white needles, m.p. 62° (others have reported the same m.p.[5,10]).

Anal. Calcd. for $C_{10}H_{13}O_3N$: N, 7.17. Found: N, 7.14, 7.20.

Phenylserine Ethyl Ester.—I ethyl ester hydrochloride (12.3 g., 0.050 mole) was treated in the manner described for preparation of I methyl ester to give 9.9 g. (95% yield) of crude I ethyl ester, m.p. 82–83°, as white plates. Recrystallization from hot cyclohexane steadied the m.p. to 83° (other values reported are 82–84°,[10] 84°[4] and 86°[5]).

Anal. Calcd. for $C_{11}H_{15}O_3N$: N, 6.69. Found: N, 6.53, 6.65.

Phenylserinol.—In an oven-dried, all-glass assembly consisting of a 500-ml. three-necked flask fitted with an oil-sealed Hershberg stirrer and Soxhlet extraction unit surmounted by a reflux condenser with attached drying tube, 5.93 g. (*ca.* 0.16 mole) of finely powdered lithium aluminum hydride was suspended in 250 ml. of sodium-dried ether. After a Soxhlet cup lined with No. 1 Whatman paper and containing 10.46 g. (0.0500 mole) of I ethyl ester had been placed in the extraction chamber, agitation and heating over a water-bath were initiated in such a way that siphoning occurred at roughly four-minute intervals. Hydrogen evolution diminished after 75 minutes. The water-bath was removed after two hours and, to destroy excess lithium aluminum hydride, ten 1.0-ml. portions of water were introduced *via* the Soxhlet cup, with allowance for two siphonings between additions.[33] A further 100 ml. of ether was added to compensate for evaporation loss. After the deposit adhering to the flask wall had been loosened with a stiff wire, refluxing with agitation was effected for a further half-hour, then 250 ml. of 10% aqueous sodium hydroxide was added, and mixing was continued for five minutes longer. The basic emulsion was cleaved in a separatory funnel, and the aqueous layer was extracted with five more 250-ml. portions of ether. The combined ether extracts were concentrated under reduced pressure to a straw-colored gum, which was taken up in 37 ml. of hot ethyl acetate and treated with 25 ml. of cyclohexane to produce a faint turbidity. After cooling at 5° for three hours, 4.12 g. (49% yield) of phenylserinol was obtained as white microcrystals, m.p. 88–89°, unchanged by recrystallization from ethyl acetate–cyclohexane. Six more extractions of the aqueous layer with 250-ml. portions of ether were carried out, and the combined ether extracts were treated as before to give a further 2.16 g. (26% yield) of phenylserinol, m.p. 88–89°. The ethyl acetate–cyclohexane filtrates were worked up to give 0.12 g. (1.4% yield) of less pure phenylserinol, m.p. 86–88°. DL-*Threo*-1-phenyl-2-amino-1,3-propanediol has been reported to melt at 86–87°.[21]

Allophenylserine Ethyl Ester Hydrochloride.—Material recovered from ethanol washings and recrystallization filtrates of the crude product of glycine–benzaldehyde condensation (54.4 g.) was suspended in 540 ml. of absolute ethanol and treated with a vigorous stream of dry hydrogen chloride for four hours. Complete solution occurred during 20 minutes, and sufficient heat was generated to induce gentle refluxing. As temperature gradually declined, precipitation of fine white crystals set in.

After refrigeration at 5° for three hours, filtration, washing with cold ethanol, then ether, and drying at 50–60°, 24.7 g. (34% yield) of II ethyl ester hydrochloride, m.p. 175–176° (dec.), was obtained as glittering white flakes. After two recrystallizations from absolute ethanol, the compound decomposed at 176°. M.p. 186° has been reported recently for "*erythro*-β-phenylserine ethyl ester hydrochloride."[29]

Anal. Calcd. for $C_{11}H_{16}O_3NCl$: N, 5.70; Cl, 14.43. Found: N, 5.73, 5.77; Cl (gravimetric), 14.36, 14.39, 14.40.

Concentration of the ethanolic hydrogen chloride filtrate and treatment with ether led to a 11.9 g. (16% yield) second crop, m.p. 140–150°, and a 12.6 g. (17% yield) third crop, m.p. 135–141°, of mixed ethyl ester hydrochlorides.

Allophenylserine Ethyl Ester.—II ethyl ester hydrochloride (12.3 g., 0.050 mole) was treated in ether with ammonia gas, as for I methyl ester. After removal of ammonium chloride, evaporation of the ether solution gave 9.6 g. (92% yield) of white needles, m.p. 85–86°. Recrystallization from 250 ml. of cyclohexane led to 8.7 g. (91% recovery) of small flakes, m.p. 83–84°. A mixture of I and II ethyl esters melted in the range 63–71°. M.p. 81–83° has been reported recently for "*erythro*-β-phenylserine ethyl ester."[29]

Anal. Calcd. for $C_{11}H_{15}O_3N$: N, 6.69. Found: N, 6.64, 6.66.

Allophenylserinol.—By using the Soxhlet technique described for preparation of phenylserinol, 4.18 g. (0.20 mole) of II ethyl ester was reduced with 2.28 g. (0.060 mole) of lithium aluminum hydride. The reaction mixture, quenched with 10 ml. of water in the manner described earlier, was filtered through a Soxhlet extraction cup, and the filtrate with a further 100 ml. of ether was used to extract the insoluble residue in the standard Soxhlet manner for one hour. The ether extract was concentrated under reduced pressure and the yellow oil taken up in 10 ml. of ethyl acetate. Crystallization commenced upon addition of 0.25 ml. of cyclohexane, 0.25 ml. of chloroform and 0.5 ml. of Skelly D. By overnight storage at 5°, filtration, cold ethyl acetate washing and drying at 50–60°, 0.98 g. (30% yield) of allophenylserinol was obtained as glittering white thin plates, m.p. 104–105°. Concentration of the filtrate under reduced pressure, solution in chloroform and precipitation with Skelly D gave 0.14 g. (4% yield) of a less pure product, m.p. 99–102°. DL-*Erythro*-1-phenyl-2-amino-1,3-propanediol has been reported to melt at 104–105°.[21]

A second Soxhlet extraction of the reduction residue with 200 ml. of ether for two hours led to a first crop of 0.47 g. (14% yield), m.p. 105–106°, and a second crop of 0.19 g. (6% yield), m.p. 102–104°. A third extraction of the residue, by thorough mixing with 150 ml. of ether, filtration, and processing of the extract as before, yielded a further 0.20 g. (6% yield), m.p. 103–104°. Only a small amount of gum was obtained by further extraction of the reduction residue with hot chloroform.

Allophenylserine (II).—II ethyl ester hydrochloride (9.828 g., 0.0400 mole) was hydrolyzed by addition of 41.00 ml. of 1.95 *N* (0.0800 mole) sodium hydroxide and 2.0 ml. of ethanol, with standing at room temperature and intermittent shaking during 90 minutes. When the clear solution was treated with 3.37 ml. (*ca.* 0.040 mole) of concentrated hydrochloric acid (*p*H *ca.* 5), rapid crystallization occurred. After overnight storage at 5°, filtration, washing with ice-water and drying in air, 5.583 g. (77% yield) of II was obtained as a mat of needles, unchanged by subsequent desiccation over Anhydrone at 50–60° and *ca.* 0.1 mm. during 16 hours. Upon recrystallization from water, the same crystal form was obtained.

Anal.[34] Calcd. for $C_9H_{11}O_3N$: C, 59.66; H, 6.09; N, 7.73. Found: C, 59.36; H, 5.96; N, 7.64.

Samples from different preparations decomposed in the range 189–193°, depending on heating rate and initial bath temperature. No depression was noted upon admixture with I. M.p. 260° was reported recently for "*erythro*-β-phenylserine."[29]

Solubilities of Diastereomeric Ethyl Ester Hydrochlorides.—Solubilities of these compounds in absolute ethanol were estimated approximately from observations made in the course of recrystallization operations. With dioxane (refluxed and distilled over sodium) and acetone (distilled from

(32) A. C. Davis and A. L. Levy, *J. Chem. Soc.*, 3479 (1951).

(33) In another run, when water was added directly to the reaction mixture, flames and sparks flickered through the ether slurry, with carbonized areas appearing on the flask wall. The danger of violent explosion, if air be present under such circumstances, is noteworthy. In runs in which ether saturated with water was used to destroy excess reductant, decomposition proceeded quietly.

(34) C, H analysis by Clark Microanalytical Laboratory, Urbana, Illinois.

potassium permanganate and dried over potassium carbonate), solutions containing excess solute were held at the specified temperatures with frequent shaking for at least one hour, after which measured volumes of clear supernatant were withdrawn, evaporated to dryness at 100° and the residues weighed. A different approach was necessary with I ethyl ester hydrochloride in dioxane at 95°, since its solubility was so great that high viscosity of the solution precluded accurate volumetric transfer. In this case, weighed portions were added to a measured volume of solvent until a trace of undissolved material still remained one hour later. Results are shown in Table III.

Effect of Phenylserine Ethyl Ester Hydrochloride on Solubility of Allophenylserine Ethyl Ester Hydrochloride in Acetone.—I ethyl ester hydrochloride (1.000 g.) was dissolved in 8.0 ml. of dry acetone by refluxing. II ethyl ester hydrochloride (0.100 g.) was added, and refluxing was continued for five minutes. The hot mixture was filtered, 1.0 ml. of cold dry acetone being used to complete transfer of the undissolved allo compound to the filter funnel. Drying gave 0.026 g. and 0.030 g. of II ethyl ester hydrochloride undissolved in two such runs, m.p. 176° (dec.).

AMES, IOWA

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

Stereochemistry of the β-Phenylserines: Improved Preparation of Allophenylserine[1]

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Paper chromatography was used to study preparation of phenylserine and allophenylserine by condensation of benzaldehyde and glycine. The isomers were produced in comparable quantities with a one-hour condensation period; the proportion of allophenylserine decreased sharply with longer reaction time. Allophenylserine forms a hemihydrate, and a poorly soluble addition compound with dioxane. The latter was used to separate allophenylserine from phenylserine. The hydrochlorides, the methyl, ethyl, *n*-propyl and *i*-propyl ester hydrochlorides, and the corresponding esters of phenylserine and of allophenylserine were prepared. Threonine and allothreonine were separated on paper chromatograms under the same conditions as phenylserine and allophenylserine.

The main product from the condensation of benzaldehyde and glycine under alkaline conditions is ordinarily phenylserine (I): some allophenylserine (II) is also formed.[3] Paper chromatography has been studied as a means of controlling preparation of the latter. A typical papergram prepared by the ascending technique[4] is illustrated in Fig. 1.

The upper layer from a mixture, by volume, of 50% *n*-butanol, 6.25% acetone, 6.25% concentrated ammonium hydroxide and 37.5% water was used for development. The difference between R_F values of I and II diminished when other levels of acetone or concentrated ammonia were used. I and II were not separated when either acetone or ammonia was omitted, when 5% sodium hydroxide or pyridine was substituted for ammonium hydroxide, or *n*-butyl ethyl ketone for acetone. A less satisfactory separation was obtained using the upper layer from a mixture of equal parts of *n*-butanol and 5% hydrochloric acid.[5]

The solvent mixture used to resolve I and II also proved effective for the separation of threonine and allothreonine. In this case, no attempt was made to vary the ratio of solvent constituents in order to attain maximum difference in R_F values. Slow separation of these diastereomers on paper by *n*-butanol–diethylamine–water has been described.[6]

Paper chromatography was qualitatively useful for solutions containing 0.01–2.5% (w./v.) of I or II. One part (0.2γ) of one diastereomer could be detected in the presence of 250 parts (50γ) of the other. The range 0.05–0.2% was preferred for quantitative estimates, which were obtained by visually comparing spot areas from solutions of unknown with those of known concentration.

The German patent[7] method of condensing glycine and benzaldehyde was then reinvestigated. The condensation cakes obtained from various batches were allowed to stand at room temperature for different periods of time prior to acidification. The proportions of I and II in the resulting crude products, aqueous filtrates and ethanol washings were determined by paper chromatography. Typical results are presented in Table I. With a one hour condensation period, II was formed to an extent comparable to I, but with increasing condensation time, the level of II declined sharply. With a 24-hour interval, combined yield of I and II was maximal, but the small quantity of II present remained in the aqueous filtrate, or was eluted during hot ethanol washing of the crude product. On the basis of these findings, II can now be prepared readily.[8]

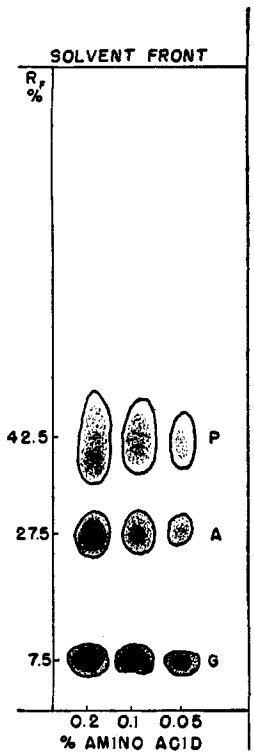

Fig. 1.—P, Phenylserine; A, allophenylserine; G, glycine.

(1) Work supported by the Industrial Science Research Institute of Iowa State College. Presented at the Northwest Regional Meeting of the American Chemical Society, Corvallis, Ore., June 20, 1952.

(2) From the Ph.D. dissertation of Kenneth N. F. Shaw, 1951.

(3) K. N. F. Shaw and S. W. Fox, THIS JOURNAL, **75**, 3417 (1953).

(4) R. J. Williams and H. Kirby, *Science*, **107**, 481 (1948).

(5) Separation of the *p*-nitrophenylserines on paper by *n*-butanol–formic acid was reported recently: D. O. Holland and P. A. Jenkins, *Chemistry and Industry*, 1092 (1951).

(6) T. L. Hardy and D. O. Holland, *ibid.*, 855 (1952).

(7) Ges. für Kohlentechnik, m.b.H., German Patent 632,424 (July 8, 1936).

(8) Since this work was completed, preparation of II has been reported from ethyl benzoylacetate[9] and its α-oximino derivative.[10,11]

(9) W. A. Bolhofer, THIS JOURNAL, **74**, 5459 (1952).

(10) Y. Chang and W. H. Hartung, *ibid.*, **75**, 89 (1953).

(11) I. Elphimoff-Felkin and H. Felkin, *Compt. rend.*, **232**, 241 (1951).