The catechol amines are rather insoluble in most organic solvents and are, furthermore, firmly adsorbed on the low-loaded columns used in this work. Epinephrine and levarterenol were, therefore, converted to the triacetyl derivatives by treatment of an aqueous solution with acetic anhydride and sodium bicarbonate (16). Gas chromatographic analysis of triacetylepinephrine produced three peaks indicating that decomposition had taken place. This decomposition was probably due to the free alcoholic hydroxyl group which in the flash heater caused alcoholysis and transesterification resulting in a mixture of diacetyl-, triacetyl-, and tetraacetylepinephrine. Similar decomposition reactions have been observed by gas chromatography of acetylated morphine (19). Attempts to acetylate epinephrine completely did not entirely eliminate this problem. However, if the secondary alcohol group of triacetylepinephrine was converted to a trimethylsilyl ether, the resulting product gave a single, symmetrical peak. Levarterenol, after acetylation and treatment with hexamethyldisilazane, could be differentiated from epinephrine on the basis of the retention time, Table I.

TABLE III.—RETENTION TIMES OF SOME PHENOLIC AMINES ON FLUOROSILICONE FLUID, QF 1-0065[a]

Compound	—Retention Time, min.— Base[b]	Acetone Deriv.
Metaraminol	...	6.7
Phenylephrine	...	5.7
Synephrine	...	5.7

[a] Column, 6 ft. × 3 mm. i.d.; 2.8% QF 1-0065 (Dow Corning) on Chromosorb, 60–80 mesh; column temp., 138°; inlet pressure 30 lb. p.s.i. [b] No elution peaks were observed for the free amines.

CONCLUSION

With the exception of phenylephrine and synephrine, all the sympathomimetic amines studied could be separated and identified. The reaction with ketones was very useful as a means of separating and identifying certain amines which followed each other very closely as the free bases. The ketone derivatives, furthermore, gave nontailing peaks that should be suitable for quantitative work. Certain phenolic amines were difficult to gas chromatograph on low-loaded columns because of adsorptive effects. The monophenolic amines investigated gave good chromatograms as the ketone derivatives. The catechol amines gave best results by acetylation and treatment with hexamethyldisilazane prior to the gas chromatographic analysis.

REFERENCES

(1) Ahlquist, R. F., in Drill, V. A., "Pharmacology in Medicine," 2nd ed., Blakiston, New York, N.Y., 1958, p. 378.
(2) Graf, E., *Pharmazie*, **5**, 108(1950).
(3) Fischer, W. H. A., and Plein, E. M., THIS JOURNAL, **44**, 313(1955).
(4) Feigl, F., and Silva, E., THIS JOURNAL, **47**, 460(1958).
(5) Chatten, L. G., and Levi, L., *Anal. Chem.*, **31**, 1581 (1959).
(6) Wickstrom, A., and Salvesen, B., *J. Pharm. Pharmacol.*, **4**, 631(1952).
(7) Pohloudek-Fabini, R., and Koenig, K., *Pharmazie*, **13**, 131(1958).
(8) Koenig, K., and Pohloudek-Fabini, R., *ibid.*, **15**, 70 (1960).
(9) Beckett, A. H., Beaven, M. A., and Robinson, A. E., *J. Pharm. Pharmacol.*, **12**, (Suppl.), 203(1960).
(10) Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **110**, 315 (1954).
(11) Williams, R. T., "Detoxification Mechanisms," 2nd ed., John Wiley and Sons, Inc., New York, 1959, p. 137.
(12) Krivulka, R. L., "The Separation and Identification of Sympathomimetic Amines from Biological Material," Thesis, University of California, 1962.
(13) Brochmann-Hanssen, E., and Svendsen, A. B., THIS JOURNAL, **51**, 393(1962).
(14) Bohemen, J., Langer, S. H., Perrett, R. H., and Purnell, J. H., *J. Chem. Soc.*, **1960**, 2444.
(15) Horning, E. C., Moscatelli, E. A., and Sweeley, C. C., *Chem. Ind. (London)*, **1959**, 751.
(16) "United States Pharmacopeia" 16th rev., Mack Publishing Company, Easton, Pa., 1960, p. 263.
(17) Bergel, F., and Lewis, G. E., *Chem. Ind. (London)*, **1955**, 774.
(18) Bergel, F., Lewis, G. E., Orr, S. F. D., and Butler, J., *J. Chem. Soc.*, **1959**, 1431.
(19) Brochmann-Hanssen, E., and Svendsen, A. B., THIS JOURNAL, in press.

Synthesis of Diphenic Acid Derivatives

By WILLIAM D. ROLL† and GUSTAV E. CWALINA

Five new derivatives of diphenic acid were prepared for the purpose of studying their antibacterial, antihyperglycemic, antispasmodic, and/or local anesthetic activity: *o,o'*-bis(2-nitro-1,3-dihydroxypropyl)biphenyl disodium salt; *o,o'*-bis(3-benzoxy-2-benzamido-1-hydroxypropyl)biphenyl; *o,o'*-bis(β-dichloroacetamidoethyl)diphenate; N,N'-bis(benzenesulfonyl)-*o,o'*-diphenoylurea, and *m,m'*-bis(N-carboxymethyl)diphenamide.

VARIOUS types of derivatives of diphenic acid were synthesized for the purpose of making them available for pharmacological testing as possible antibacterial, antihyperglycemic, antispasmodic, and/or local anesthetic agents.

Roberts and Johnson (1) reported that diethylaminoethyl diphenate and diethylaminoethyl imidoester of diphenanilide possessed local anesthetic activity. Case and Koft (2) synthesized a similar compound, diethylaminoethyl 5,5-diaminodiphenate, which had marked local anesthetic activity. A number of substituted amine esters and amides of diphenic acid were prepared by Demers and Jenkins (3) for evaluation as possible antispasmodics and antihistamines. Compound VII of our derivatives contains an amide-ester linkage.

Woolley (4) has suggested that analogs of phenylalanine might function as antimetabolites

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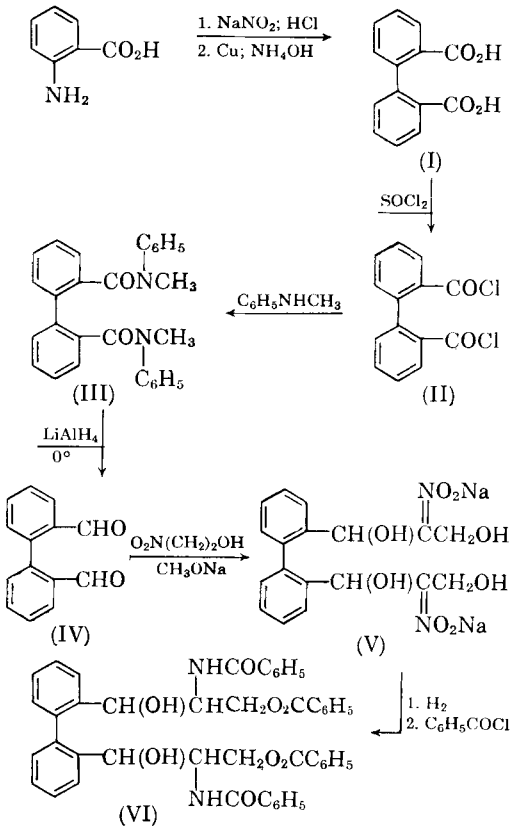
by interfering with the enzymatic transformations which are undergone by an essential metabolite probably related to phenylalanine. Such analogs might inhibit the incorporation of phenylalanine into protein. Structural analogs of the amino acid glycine might also serve as specific inhibitors of protein synthesis. Such compounds may have antibacterial activity since protein synthesis is a process fundamental to the growth of living organisms. Compounds V and VI can be regarded as analogs of phenylalanine, differing from the amino acid in four positions. Compound XI is an amide derivative of glycine. The report (5) of the oral antidiabetic compound tolbutamide[1] stimulated the synthesis of the sulfonylurea derivative of diphenic acid. Compound VIII has structural features similar to tolbutamide.

The synthetic procedure used for the preparation of these compounds may be briefly outlined as follows: *o,o'*-diphenic acid, I, synthesized by the procedure described by Atkinson and Lawler (6), was converted to *o,o'*-diphenoyl chloride, II, (7). In order to introduce an alkanol side chain, the acyl chloride was converted in a step-wise manner to *o,o'*-bis(N-methyl)diphenanilide, III, (8), *o,o'*-diphenaldehyde, IV, (8) and *o,o'*-bis(2-nitro - 1,3 - dihydroxypropyl)biphenyl disodium salt, V. The nitroalcohol was reduced with hydrogen and palladous oxide and isolated as the amide-ester *o,o'*-bis(3-benzoxy-2-benzamido-1-hydroxypropyl)biphenyl, VI.

One ester of diphenic acid was prepared by treating II with β-dichloroacetamidoethanol to form *o,o'*-bis(β-dichloroacetamido-ethyl)diphenate, VII. Two amides of diphenic acid, N,N'-bis(benzenesulfonyl) - *o,o'* - diphenoylurea, VIII, and *m,m'*-bis(N-carboxymethyl)diphenamide, XI, were synthesized by reacting II with benzenesulfonylurea (9) and *m,m'*-diphenic acid, X, with thionyl chloride and glycine.



EXPERIMENTAL

The sequence of synthetic reactions is shown by the diagrams in the text.

***o,o'*-Diphenic Acid, I.**—The procedure used for the synthesis of diphenic acid was that described in "Organic Syntheses" (6).

***o,o'*-Diphenoyl Chloride, II.**—This known compound was prepared from I and thionyl chloride. Yields varied from 89–98% and the product melted at 94–95° (7).

***o,o'*-Bis(N-methyl)diphenanilide, III.**—N-Methylaniline, 30.9 Gm. (0.29 mole), dissolved in 150 ml. of dry benzene, was added in 5-ml. portions over a 1-hr. period to a well stirred solution of 20 Gm. (0.072 mole) of II in 200 ml. of dry benzene. The reaction mixture was stirred for an additional 15 minutes after all of the amine had been added. The reaction mixture, after filtration, was washed with 3 *N* hydrochloric acid until the aqueous phase was colorless and then with distilled water until the aqueous phase was no longer acidic to litmus. The benzene layer was dried over anhydrous sodium sulfate for 24 hours, filtered, and the solvent removed *in vacuo*. The crude residue was recrystallized from acetone-water. The average yield of product (four runs) was 24.2 Gm. (80%); m.p. 160–161° (8).

***o,o'*-Diphenaldehyde, IV.**—In a 1-L. three-neck flask equipped with a mercury-sealed stirrer, a reflux condenser equipped with a calcium chloride tube, and dropping funnel was placed a solution containing 50 Gm. (0.119 mole) of III in 450 ml. of anhydrous tetrahydrofuran. This solution was cooled to 0° and a previously prepared solution containing 2.5 Gm. (0.066 mole) of lithium aluminum hydride in 65 ml. of anhydrous ether was placed in the dropping funnel. The hydride was added dropwise with rapid stirring over a 3-hr. interval. Stirring was continued for 5 additional hours while maintaining the temperature of the reaction mixture at 0°. At the end of this period, the cooling bath was removed and the excess lithium aluminum hydride was decomposed with an ice-cold solution of 5% hydrochloric acid. The reaction mixture was filtered into a separator and extracted with 100 ml. of ether. The ether solution was then washed successively with water, 5% hydrochloric acid, and water. The ether layer was dried over anhydrous sodium sulfate for 24 hours, filtered, and the solvent removed *in vacuo*. The residue weighed 20 Gm.

[1] Marketed as Orinase by The Upjohn Co.

The 2,4-dinitrophenylhydrazone of a portion of the residue was prepared; m.p. 168–169°. The reported (8) melting point is 168°.

***o,o'*-Bis (2-nitro-1,3-dihydroxypropyl)biphenyl Disodium Salt, V.**—In a 300-ml. round-bottom flask equipped with a mercury-sealed stirrer and 125-ml. dropping funnel, was placed a solution containing 20.0 Gm. (0.095 mole) of IV in 75 ml. of absolute methanol and 18.6 Gm. (0.204 mole) of 2-nitroethanol. The mixture was stirred and cooled to −10°. A freshly prepared solution of sodium methoxide containing 4.4 Gm. (0.190 mole) of sodium in 45 ml. of absolute methanol was cooled to −10° and transferred to the dropping funnel. The base was added in a dropwise manner over 30 minutes. The reaction mixture was stirred for 1 hour after all of the base had been added. The product was filtered, washed first with anhydrous ether, and then with three 10-ml. portions of cold absolute methanol. A yield of 36.5 Gm. (88%) was obtained. A portion of the product was recrystallized from a cold solution of acetone and water and analyzed for sodium (10).

Anal.—Calcd. for $C_{18}H_{18}N_2Na_2O_8$: Na, 10.55. Found: Na, 10.55, 10.56.

***o,o'* - Bis(3 - benzoxy - 2 - benzamido - 1 - hydroxypropyl)biphenyl, VI.**—A 35-Gm. quantity of *o,o'*-bis(2-nitro-1,3-dihydroxypropyl)biphenyl disodium salt, V, was dissolved in 120 ml. of glacial acetic acid in a Parr reduction bottle. Palladous oxide, 1 Gm., was added and the mixture was reduced at 50 p.s.i. for 24 hours. The reaction mixture was filtered and the filtrate heated *in vacuo* at 40°. The residue was dissolved in 250 ml. of water and extracted with ether. The aqueous phase was made strongly alkaline with sodium hydroxide.

A portion of the strongly basic solution was shaken with 45 Gm. of benzoyl chloride and 100 ml. of chloroform for 20 minutes in a separator. The mixture was allowed to stand for 12 hours. The chloroform layer was drawn off and separated. A white, gummy residue remained which, when stirred repeatedly with fresh portions of dry petroleum ether, solidified to a white crystalline solid. The crude product was recrystallized from ethanol-water yielding 10.5 Gm.; m.p. 85–87°.

Anal.—Calcd. for $C_{46}H_{40}N_2O_8$: N, 3.74; sapon. equiv., 187. Found: N, 3.80, 3.81; sapon. equiv., 173.

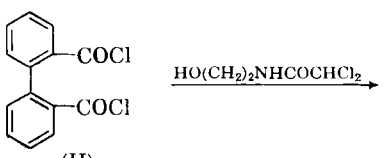
***o,o'* - Bis(β - dichloroacetamidoethyl)diphenate, VII.**—In a 250-ml. Erlenmeyer flask was placed 21.45 Gm. (0.15 mole) of methyl dichloroacetate. To this, with constant shaking, was added 9.15 Gm. (0.15 mole) of β-ethanolamine. The reaction mixture became warm and the heretofore colorless solution became bright red. When it had cooled to room temperature it was transferred to a 400-ml. beaker and allowed to crystallize at room temperature. The product, β-dichloroacetamidoethanol, was filtered and recrystallized from chloroform. It weighed 24.5 Gm. (95%); m.p. 85–86°.

To a cold (0°) well-stirred mixture of 2.79 Gm. (0.01 mole) of II, 50 ml. of dry benzene and 3 ml. of anhydrous pyridine there was added 3.42 Gm. (0.02 mole) of β-dichloroacetamidoethanol obtained above in small portions over a 30-min. period. The reaction mixture was stirred an additional 10 minutes. A heavy gum formed in the bottom of the reaction vessel. The supernatant liquid was decanted and the gum was washed with anhydrous ether. The gum was dissolved in 50 ml. of 95% ethanol and filtered. To the clear, yellow filtrate was added about 1–2 ml. of distilled water with shaking. The flask was allowed to stand overnight. The next day, the pale yellow crystals were removed on a Buchner funnel, dried, and recrystallized from ethanol, yielding 3.85 Gm. (70%) of product; m.p. 111–112°.

Anal.—Calcd. for $C_{22}H_{20}Cl_4N_2O_6$: N, 5.09; Cl, 25.82. Found: N, 5.08, 5.08; Cl, 25.76, 25.83.

N,N' - Bis(benzenesulfonyl) - *o,o'* - diphenoylurea, VIII.—In a 50-ml. Erlenmeyer flask was placed 1.4 Gm. (0.005 mole) of II and 2.0 Gm. (0.01 mole) of benzenesulfonylurea and the contents were thoroughly mixed. The flask was placed in an oil bath which was heated to and maintained at 130° for 2.5 hours. At this point, the bath temperature was raised to 160° and was kept between 160–170° for 6.5 hours. The flask was removed from the heating bath and allowed to come to room temperature. The crude product was recrystallized from absolute alcohol. A yield of 1.9 Gm. (64%) of product was obtained; m.p. 124–126°.

Anal.—Calcd. for $C_{28}H_{22}N_4O_8S_2$: N, 9.40; S, 10.74. Found: N, 9.28, 9.28; S, 10.57, 10.60.



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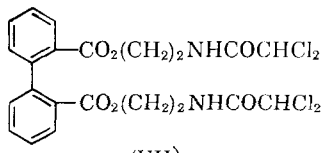

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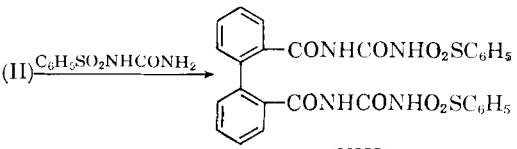
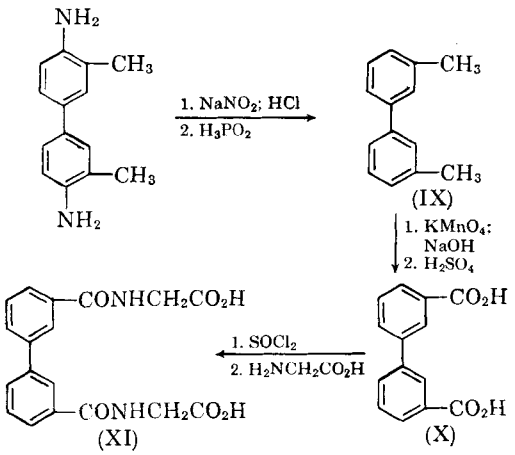


(VIII)

***m,m'* - Bis(N - carboxymethyl)diphenamide, XI.**—*m,m'*-Bitolyl, IX, was prepared by the method described by Kornblum (11). The yield was 88% and the product distilled at 134–135° (6 mm.). A 4-Gm. quantity (0.022 mole) of IX, 15.8 Gm. (0.1 mole) of potassium permanganate, 2.0 Gm. (0.05 mole) of sodium hydroxide and 420 ml. of water were heated under reflux for a period of 12 hours. At this time, the solution was filtered while still hot and the filtrate carefully acidified by the slow addition of concentrated sulfuric acid. The white precipitate, *m,m'*-diphenic acid, X, after drying, weighed 3.2 Gm. (60%); m.p. 356–358°.

One gram (0.006 mole) of *m,m'*-diphenic acid, X, was refluxed with an excess (25 ml.) of thionyl chloride for 6 hours. The excess thionyl chloride was removed by distillation. The product was washed with successive portions of anhydrous benzene and dried. It weighed 1.2 Gm. One gram (0.004 mole) of the crude product obtained, 0.75



Gm. (0.01 mole) of glycine, and 1.0 Gm. (0.025 mole) of sodium hydroxide in 50 ml. of water were refluxed for 30 minutes. The mixture was cooled and the product precipitated by the slow addition of a 5% solution of hydrochloric acid and recrystallized from ethanol, yielding 1.1 Gm. of product (78.6%); m.p. 204° (decompn.).

Anal.—Calcd. for $C_{18}H_{16}N_2O_6$: N, 7.86. Found: N, 7.88, 7.89.

SUMMARY

Five new derivatives of diphenic acid have been prepared for pharmacological testing as possible antibacterial, antihyperglycemic, antispasmodic, and/or local anesthetic activity: *o,o'* - bis(2-nitro - 1,3 - dihydroxypropyl)biphenyl disodium salt; *o,o'* - bis(3 - benzoxy - 2 - benzamido - 1-hydroxypropyl)biphenyl; *o,o'* - bis(β - dichloroacetamidoethyl)diphenate; N,N' - bis - (benzenesulfonyl) - *o,o* - diphenoylurea, and *m,m'*-bis(N-carboxymethyl)diphenamide.

REFERENCES

(1) Roberts, R. C., and Johnson, T. B., *J. Am. Chem. Soc.*, **47**, 1396(1925).
(2) Case, H. F., and Koft, E., *ibid.*, **63**, 508(1941).
(3) Demers, F. X., Jr., and Jenkins, G. L., THIS JOURNAL, **41**, 61(1952).
(4) Woolley, D. W., *J. Biol. Chem.*, **185**, 293(1950).
(5) *Chem. Eng. News*, **34**, 5512(1956).
(6) Atkinson, E. R., and Lawler, H. J., "Organic Syntheses," Coll. Vol. 1, 2nd ed., John Wiley & Sons, New York, N. Y., 1941, pp. 222–224.
(7) Nightingale, D., Heiner, H. E., and French, H. E., *J. Am. Chem. Soc.*, **72**, 1876(1950).
(8) Weygand, F., *Angew. Chem.*, **65**, 529(1953).
(9) Haack, E., U. S. pat. 2,385,571.
(10) Garratt, D. C., "The Quantitative Analysis of Drugs," 2nd ed., Philosophical Library, New York, N. Y., 1955, p. 25.
(11) Kornblum, N., "Organic Syntheses," Coll. Vol. 3, 2nd ed., John Wiley & Sons, New York, N. Y., 1955, pp. 295–299.

Analysis of Phenobarbital Elixir by an Ion Exchange and Nonaqueous Titration Procedure

By MARTIN I. BLAKE and FREDERICK P. SIEGEL

Phenobarbital elixir is analyzed by passing an aliquot through a strongly basic anion exchange resin. The phenobarbital is eluted from the column with acetic acid in ethanol. After evaporation of the solvent, the residue is dissolved in dimethylformamide and titrated with sodium methoxide in benzene-methanol. Other barbiturate elixirs are assayed similarly. Nonionic components, coloring agents, bases, most salts, and synthetic sweetening agents do not interfere.

THE ANALYSIS of barbiturate salts in a variety of dosage forms by ion exchange and nonaqueous titration was recently reported (1). Application to the assay of elixirs proved unsuccessful. Ion exchange resins have been employed for the isolation of barbiturates (2) and a variety of nonaqueous techniques have been described for determining barbiturates in dosage forms. These have been reviewed in an earlier paper (1). The usual procedure in applying a nonaqueous titration procedure to the analysis of elixirs involves extraction of the barbiturate with an organic solvent prior to titration in a nonaqueous medium.

Potentiometric titration of phenobarbital elixir with silver nitrate was reported by Mattocks and Voshall (3) and later by Bodin (4). Cohen and Lordi (5) developed an amperometric and a potentiometric titration procedure with mercuric ion for analyzing the elixir. A general review of the literature dealing with the analysis of the barbituric acids is presented by Connors (6).

The official assay for phenobarbital elixir is a gravimetric one in which the phenobarbital is extracted with chloroform. High results are usually obtained because of extraction of other chloroform soluble components. This has been noted by several workers (3, 7). In addition, the official assay is tedious and time consuming.

This report describes a procedure in which the phenobarbital is removed from the elixir by

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