hours. Immediate fractionation at 2.5 mm. gave 30.1 g. of bromoacetone in the Dry Ice trap and 6.3 g. (12%) of the self-condensation product, b.p. 56–60° (2.5 mm.), 42% bromine (42.5% when K. A. was present as per the above). The other properties of this product were the same as in the case of the former sample prepared as above.

Reactions of K. A. with 1,1,3-Trichloro-2-methyl-1-propene (I).—A mixture of 27.0 g. (0.234 mole) of K. A. and 38.6 g. (0.24 mole) of I was heated in sealed, necessarily new rigorously dried[7] Pyrex bomb tubes held at 190–200° for 4 hours. The tubes contained a high pressure of ethylene. There were isolated as products: 2.1 g. (21%) of ethyl chloride, b.p. 12–12.5°; 17.6 g. of ethyl acetate with about 4% of ethanol present, b.p. 70–80°, 13.7 g. of recovered I, mostly taken at b.p. 36–38° (0.8 mm.); 4.5 g. of ethyl 3,3-dichloro-2-methylallylacetate (III), b.p. 67–73° (1.1 mm.), of which an analytically pure cut had b.p. 66–68° (0.4 mm.), sapn. equiv. 216 (calcd. 211), 33.81% Cl (calcd. 33.79% Cl), 20.8% ethoxy (calcd. 21.38%).

K. A. with Allyl Chloride (II).—A mixture of 0.157-mole amounts of these substances was heated and handled as above for I. After venting the ethylene, fractionation of the reaction mixture gave 1.03 g. (22%) of ethyl chloride, b.p. 12–12.5°; 6.5 g. of allyl chloride, b.p. 43–45°; 8.0 g. of 95% ethyl acetate and 5% ethanol, b.p. 70–79°; and 3.2 g. (35.2%) of ethyl allylacetate, b.p. 78–80° (80 mm.), sapn. equiv. 129.3 (calcd. 128.0). 35.4% ethoxyl (calcd. 35.2%), and b.p. 140–142°.[13]

Reaction of N-Chlorosuccinimide with K. A.—A solution of 32.1 g. (0.241 mole) of N-chlorosuccinimide in 190 ml. of absolute carbon tetrachloride was refluxed at 76–77° while K. A. (28.0 g., 0.241 mole) was added dropwise (two hours). Ethylene was evolved with extreme frothing. It was identified by reaction with permanganate and with bromine in carbon tetrachloride; no ethyl chloride could be detected. After further reaction at 76° for 2.5 hours and at 35° for 12 hours, the reaction mixture was concentrated with occasional removal of the precipitated succinimide. A total of 21.0 g. (90.1%), m.p. 123–124°, was collected. Fractionation of the remaining liquid gave 15.2 g. of a mixture of products, b.p. 39–41° (0.25 mm.). Fractionation of a 10.1-g. portion of this gave 5.7 g. (29%) of analytically pure ethyl chloroacetate, b.p. 145–146°, 27.9% Cl (calcd. 28.01%), and 36.5% ethoxyl (calcd. 36.8%). It was converted to phenoxyacetic acid, m.p. 97.9–98.7°, no depression on admixture with known phenoxyacetic acid. From another experiment, 18.1 g. of distillate, b.p. 70–72° (5.0 mm.) was obtained after removal of succinimide. Redistillation of this distillate gave 13.2 g. (44%) of ethyl chloroacetate, b.p. 144–147°.

(13) F. Zeidler, *Ann.*, **187**, 39 (1877).

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A Simplified Procedure for the Synthesis of 2,4-Dinitrophenyl(DNP)-amino Acids

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The original method employed by Sanger[3] for the synthesis of DNP amino acids has been unmodified by later workers in the field of end group and amino acid analysis. The procedure comprises shaking the amino acid with a twofold excess of 1-fluoro-2,4-dinitrobenzene (FDNB) and an equal weight of sodium bicarbonate in 67% ethanol (by volume) for two hours at room temperature, followed by evaporation of the ethanol, dilution with water, and extraction of excess FDNB with ether. Acidification then yields the required DNP amino acid.

(1) Deceased, August 21, 1954.

(2) This manuscript was revised from the notes of the late Dr. Levy.—C. H. Li.

(3) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

It has been found that several advantages result from working in an aqueous solution at a somewhat higher *p*H (9.0) and a slightly elevated temperature (40°) and from employing only an equivalent amount of FDNB: a more rapid reaction can be achieved, ethanol evaporation and extraction of excess FDNB can be eliminated and a purer product results with greater economy of reagents. By means of this simplified procedure,[4] we have prepared in crystalline form DNP derivatives of the known amino acids, including DNP-L-glutamic acid and bis-DNP-L-cysteine. The syntheses of these latter two derivatives have not been reported hitherto. Crystalline DNP-DL-methionine sulfoxide, DNP-DL-methionine sulfone and DNP-DL-alanylglycylglycine also have been prepared.

The time needed for reaction varies for different amino acids but rarely exceeds 1 hour (at 40°), except in the case of complete substitution of the imidazole group of histidine, which requires a considerably longer time, so that more than two equivalents of FDNB are needed. In general, DNP-DL-amino acids crystallized more readily than the corresponding L-derivatives, particularly in the cases of glutamic acid, methionine, leucine and tyrosine.

Experimental[5]

DNP-L-leucine.[5]—L-Leucine (Schwarz Laboratories, Inc., Mt. Vernon, N. Y., 1.30 g.) and sodium carbonate (anhydrous, 2.0 g.) were dissolved in 40 ml. of water at 40°. FDNB (Eastman Kodak Co., 1.85 g.) was introduced, and the mixture was stirred vigorously by means of a magnetic stirrer, the temperature being maintained at about 40°. The finely divided suspension of FDNB disappeared after about 30 minutes indicating that the reaction was complete. Acidification (concentrated hydrochloric acid, 3 ml.) of the resulting orange solution yielded DNP-L-leucine, which crystallized on rubbing (yield 2.89 g., 97%, the remaining 3% appearing as dinitrophenol). It was recrystallized from carbon tetrachloride and subsequently from aqueous acetic acid, as yellow needles, m.p. 101° (uncor.) and $[\alpha]^{23}D$ +56.6° (1% $NaHCO_3$).

Anal. Calcd. for $C_{12}H_{15}O_6N_3$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.50; H, 4.99; N, 13.98.

It is perhaps worth mentioning that L-leucine from another commercial source yielded crystals only with great difficulty; synthetic L-leucine, however, readily afforded a crystalline DNP derivative.

DNP-L-Glutamic Acid.—By the method described above, 2.9 g. of L-glutamic acid, 3.7 g. of DNFB and 6.0 g. of Na_2CO_3 yielded 5.08 g. (81%) of the product after recrystallization from ethyl acetate–chloroform; m.p. 134–136°, $[\alpha]^{23}D$ −18.48° (1% $NaHCO_3$).

Anal. Calcd. for $C_{11}H_{11}N_3O_8$: C, 42.17; H, 3.51; N, 13.42. Found: C, 42.07; H, 3.66; N, 13.25.

DNP-DL-Methionine Sulfoxide.—By the same procedure 1.65 g. of DL-methionine sulfoxide, 1.85 g. of DNFB and 2 g. of Na_2CO_3 yielded 3.18 g. (96%) of the product, recrystallized from hot ethanol; m.p. 184.5° dec.

(4) Greater control can be effected by carrying out the reaction at *p*H 9.0 (glass electrode), and adding 2 *N* sodium hydroxide intermittently to maintain the *p*H at this value. The rate of addition of alkali then serves to indicate the rate of the reactions and results in the consumption of the theoretical 2 equivalents per amino group, provided that the alkali needed to titrate the amino acid from isoelectric condition to *p*H 9.0 is included.

(5) Since this manuscript was prepared for publication, Rao and Sober (THIS JOURNAL, **76**, 1328 (1954)) reported the preparation of DNP-L-leucine by the procedure of Sanger and gave its m.p. as 94–95° (uncor.) and $[\alpha]^{24}D$ +59.25° (4% $NaHCO_3$). The following description of its synthesis in crystalline form serves as an illustration of the method.

Anal. Calcd. for $C_{11}H_{13}N_3O_7S_1$: C, 39.88; H, 3.93; N, 12.68. Found: C, 40.20; H, 4.12; N, 12.37.

DNP-DL-Methionine Sulfone.—From 1.81 g. of DL-methionine sulfone and 1.85 g. of DNFB, 3.09 g. (89%) of the product was recrystallized from hot ethanol; m.p. 184.5° dec.

Anal. Calcd. for $C_{11}H_{13}N_3O_8S_1$: C, 38.04; H, 3.75; N, 12.10. Found: C, 38.15; H, 3.94; N, 11.97.

Bis-DNP-L-cysteine.—From 0.88 g. of L-cysteine·HCl·H_2O and 1.86 g. of FDNB, 2.29 g. (83%) of the product was obtained; it was recrystallized in CH_3COOH–H_2O and it melted with decomposition at 155–169°, $[\alpha]^{23}D$ −265.8° (1% $NaHCO_3$).

Anal. Calcd. for $C_{15}H_{11}N_5O_{10}S$: C, 39.74; H, 2.43; N, 15.45. Found: C, 39.97; H, 2.51; N, 15.50.

DNP-DL-Alanylglycylglycine.—By the same procedure, DNP peptides can be prepared in good yield. From 0.97 g. of DL-alanylglycylglycine (Nutritional Biochemical Corp., Cleveland, Ohio) and 0.89 g. of FDNB, 1.28 g. (72%) of the product was obtained after recrystallization in 50% aqueous ethanol; m.p. 205° dec.

Anal. Calcd. for $C_{13}H_{15}N_5O_8$: C, 42.28; H, 4.07; N, 18.97. Found: C, 41.37; H, 4.43; N, 18.53.

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1,2-Diphenyl-4-alkyl-3,6-diketohexahydropyridazines

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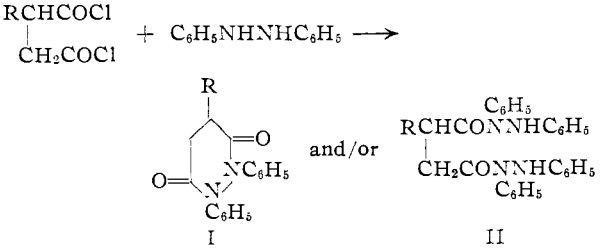
It has been reported that 1,2-diaryl-4-alkyl-3,5-diketopyrazolidines[1] possess analgesic, antipyretic, drug potentiating and anti-inflammatory activity,[1b,2] with an apparent peak of useful activity in 1,2-diphenyl-4-(*n*-butyl)-3,5-diketopyrazolidine, which is not without side-effects.[3] Because of some experience[4] with the six-membered ring structure, pyridazine, it seemed of interest to learn if the homologous 1,2-diphenyl-4-alkyl-3,6-diketohexahydropyridazines (I) possessed a pharmacological action similar to that of the corresponding diketopyrazolidines. A search of the literature revealed no compounds of this general structure. The description of the preparation of a few compounds of the desired type constitutes the subject of this paper.

Two obvious ways of preparing these compounds are (1) condensation of an α-alkylsuccinic ester with hydrazobenzene and (2) condensation of an α-alkylsuccinyl chloride with hydrazobenzene in the presence of a base. An attempt to cause a condensation of diethyl α-methylsuccinate with hydrazobenzene by heating them with sodium ethoxide at 150° resulted in a tarry mixture from which no crystalline material was obtained. When hydrazobenzene was added gradually to a mixture prepared by adding α-methylsuccinyl chloride to a solution of pyridine in ether, 1,2-diphenyl-4-methyl-3,6-diketohexahydropyridazine was formed in poor yield, although the crystalline product was easily isolated and purified. It then was found that when the order of mixing the reactants was reversed (*i.e.*, the α-methylsuccinyl chloride was added slowly to a solution of pyridine and hydrazobenzene in ether) the yield was increased to about 40%; consequently, this latter procedure was used to prepare the other compounds in the series. α-Ethylsuccinyl chloride also gave about a 40% yield of the corresponding 4-ethyl compound. α-(*n*-Propyl)-succinyl chloride gave a considerably lower yield (*ca.* 16%) and in addition there was isolated about an equal amount of a more soluble material whose analysis showed it to be the open-chain compound, α-(*n*-propyl)-N,N′,N″,N‴-tetraphenylsuccinic dihydrazide. When α-(*n*-butyl) succinyl chloride was allowed to react with hydrazobenzene under the same conditions the only crystalline product isolated was the open chain dihydrazide. We have not investigated whether this phenomenon of change in the amounts of isolated cyclic I and open chain II products, as the alkyl group increases from methyl to butyl, is caused by a change in the ease of crystallization of the products or whether the increase in bulk of the alkyl group actually hinders the ring formation.



α-(*n*-Propyl)-succinyl chloride and α-(*n*-butyl)-succinyl chloride are not reported previously in the literature; their preparation from the corresponding succinic acids and phosphorus pentachloride is described in the Experimental part of this paper.

Pharmacology.—The diketohexahydropyridazines and succinic dihydrazides herein described were administered orally to mice; in doses up to 2000 mg./kg. there were no deaths and no toxic signs. 1,2-Diphenyl-4-(*n*-propyl)-3,6-diketohexahydropyridazine and 1,2-diphenyl-4-ethyl-3,6-diketohexahydropyridazine did not demonstrate any analgesic activity in rats by the thermal radiation technique,[5] in oral doses of 500 and 200 mg./kg., respectively. They also did not show any antipyretic activity in rats, by the test procedure of Brownlee,[6] in oral doses of 400 mg./kg. We are indebted to Dr. John F. Reinhard and Miss Mary N. Lewis, of our Pharmacology Department, for these data.

The lack of pharmacological activity very probably indicates that the compounds are poorly absorbed; they are insoluble in water over a wide *p*H range. Since 1,2-diphenyl-4-(*n*-butyl)-3,5-diketo-

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(2) R. Domenjoz, *Intern. Record of Med.*, **165**, 467 (1952); A. Hemming and W. C. Kuzell, *Antibiotics and Chemotherap.*, **3**, 634 (1953); H. P. Johnson, Jr., E. P. Engleman, P. H. Forsham, M. A. Krupp, T. W. Green and A. Goldfien, *New Eng. J. Med.*, **250**, 665 (1954).

(3) S. Brandwein, *N. Y. State J. Med.*, **53**, 1577 (1953); I. H. Wood, *Brit. Med. J.*, 802 (1954); D. J. O'Brien, *ibid.*, 792; S. J. Stell and J. L. Moffatt, *ibid.*, 795.

(4) J. A. King and F. H. McMillan, THIS JOURNAL, **73**, 4911 (1951); **74**, 3222 (1952); J. Shavel, Jr., F. Leonard, F. H. McMillan and J. A. King, *J. Am. Pharm. Assoc. (Sci. Ed.)*, **42**, 402 (1953).

(5) N. Ercoli and M. N. Lewis, *J. Pharmacol. Exptl. Therap.*, **84**, 301 (1945).

(6) G. Brownlee, *Quart. J. Pharm. and Pharmacol.*, **10**, 609 (1937).