The alternative synthetic paths also yielded the same compound in the preparation of N^1-benzyl-N^1-methyl-N^5-(β-phenethyl)-biguanide hydrochloride (Table I, compound 31).

Methylbiguanide Dihydrochloride (by Catalytic Debenzylation of N^1-Benzyl-N^1-methylbiguanide Hydrochloride).—A solution of 12.0 g. (0.05 mole) of N^1-benzyl-N^1-methylbiguanide hydrochloride[3] in 120 ml. of ethanol and 50 ml. of water was treated with a suspension of palladium-on-carbon (from 1.0 g. of palladium chloride in 35 ml. of water and 0.5 ml. of 3 *N* hydrochloric acid, and 10.0 g. of carbon (Darco)) and hydrogenated at 45° under 50 lb. hydrogen pressure for 22 hours in the Parr hydrogenator. At this point two equivalents of hydrogen was absorbed. The catalyst was removed and the filtrate (strong toluene odor) was evaporated to dryness. The residue of 6.45 g. was leached with 900 ml. of acetonitrile and the insoluble portion of 5.65 g. was recrystallized (ethanol) to yield 3.23 g. of a mixture of mono- and dihydrochloride of the product. One gram of this mixture was dissolved in 20 ml. of methanol and 2.5 ml. of 3 *N* hydrochloric acid and was evaporated to dryness. The residue recrystallized (ethanol–hexane) gave 0.92 g. of product which melted at 225° dec.

Anal. Calcd. for $C_8H_{11}Cl_2N_5$: C, 19.2; H, 5.9; N, 37.2. Found: C, 19.2; H, 5.9; N, 36.5.

The dipicrate melted at 198–199°.

Anal. Calcd. for $C_{15}H_{15}N_{11}O_{14}$: C, 31.5; H, 2.7. Found: C, 31.0; H, 2.9.

Similar attempted debenzylations with compounds 20 and 26 of Table I were unsuccessful and afforded only recovery of the initial reactant.

TABLE IV

SPECTRA OF BIGUANIDES[a]

No.[b]	λ_{max}, mμ	$\epsilon \times 10^{-3}$
17	236	16.5
19	236	14.6
20	237	17.8
24	238	15.5
26	240	17.9
28	240	17.4
29	244	18.9

[a] The spectra were determined in water in the Beckman DK recording spectrophotometer, using 1-cm. cells. [b] The compounds correspond to compound numbers in Table I.

Ultraviolet Absorption Spectra.—The spectra of some of the compounds have been described in Table IV. Relative to the spectrum of β-phenethylbiguanide hydrochloride,[1] it is noted that increasing substitution in the N^1,N^5-substituted biguanides is associated with bathochromic and hyperchromic effects.

Acknowledgment.—The authors are grateful to Dr. G. Ungar for the results of the hypoglycemic tests reported herein, and to M. Blitz for the ultraviolet absorption spectra.

YONKERS 1, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XX. Diazoacetyl Analogs of Chlorambucil

BY W. A. SKINNER, HELEN F. GRAM, CAROL W. MOSHER AND B. R. BAKER

RECEIVED FEBRUARY 12, 1959

3-(*p*-Diazoacetylphenyl)-propionic acid (VI) was synthesized from methyl hydrocinnamate (I) *via* the key intermediate, 3-(*p*-glycylphenyl)-propionic acid hydrochloride (V). Attempts to synthesize 3-(*p*-diazoacetamidophenyl)-propionic acid (XV) by two different routes failed on the last step. However, the corresponding methyl ester XVI was synthesized from 3-(*p*-aminophenyl)-propionic acid (VII) *via* methyl 3-(*p*-glycylaminophenyl)-propionate hydrochloride (XIV).

Interest in diazo derivatives of amino acids has been stimulated by the discoveries that azaserine[2] (O-diazoacetyl-L-serine) and DON[3] (6-diazo-5-oxo-L-norleucine) show activity in inhibiting the growth of the Crocker Sarcoma-180 tumor.

Sarcolysine (3-{*p*-[bis-(2-chloroethyl)-amino]-phenyl}-DL-alanine, "phenylalanine mustard")[4] has been shown to be one of the more active nitrogen mustard compounds. In addition, Luck[5] has found that the two next higher homologs of phenylalanine mustard are also effective on the Cloudman malignant melanoma (S-91).

Everett, *et al.*,[6] found that a series of *p*-[bis-(2-chloroethyl)-amino]-phenylcarboxylic acids inhibit the growth of the transplanted Walker rat Sarcoma-256, the most active compound being the butyric acid derivative (chlorambucil). The methyl and ethyl esters were also active.

The hypotheis has been proposed[7] that azaserine, sarcolysine and chlorambucil might be considered members of a broad class of anticancer agents consisting of metabolites bearing an alkylating group that function by irreversible inhibition of the corresponding enzymes. This hypothesis suggests that 3-phenylpropionic acid or 4-phenylbutyric acid be considered as carrier (metabolite) groups for the diazoalkyl grouping characteristic of azaserine. This paper describes the synthesis of two such compounds, namely, 3-(*p*-diazoacetylphenyl)-propionic acid (VI) and methyl 3-(*p*-diazoacetamidophenyl)-propionate (XVI).

3 - (*p* - Diazoacetylphenyl) - propionic acid (VI) was synthesized in five steps from methyl hydrocinnamate (I). A Friedel-Crafts reaction of acetyl

(1) This program is under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, Contract No. SA-43-ph-1892. For the preceding paper in this series *cf.* C. D. Anderson, L. Goodman and B. R. Baker, paper XIX of this series, THIS JOURNAL, **81**, 3967 (1959).

(2) C. C. Stock, D. A. Clarke, H. C. Reilly, S. M. Buckley and C. P. Rhoads, *Nature*, **173**, 71 (1954).

(3) D. A. Clarke, H. C. Reilly and C. C. Stock, Abstracts of Papers, 129th Meeting, American Chemical Society, Dallas, Texas, April, 1956, p. 12-M; H. A. DeWald and A. M. Moore, THIS JOURNAL, **80**, 3941 (1958).

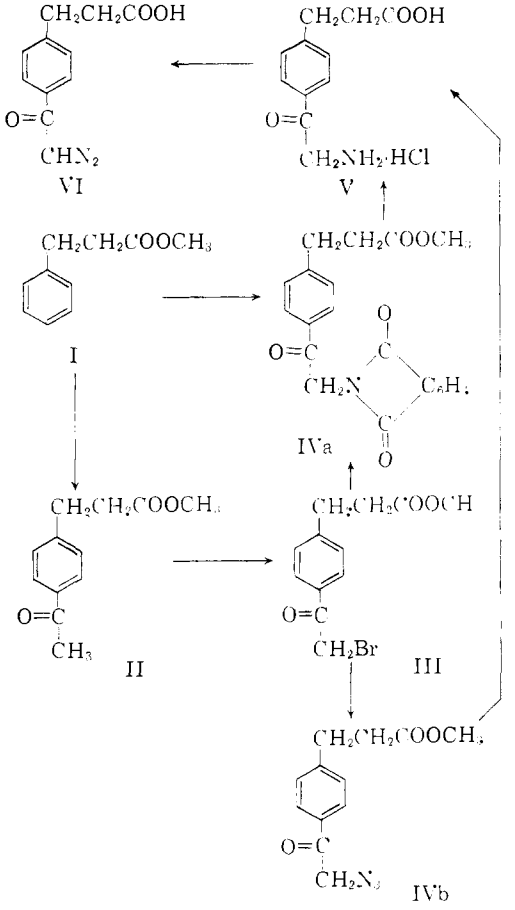
(4) F. Bergel, V. C. E. Burnop and J. A. Stock, *J. Chem. Soc.*, 1223 (1955); F. Bergel and J. A. Stock, *ibid.*, 2409 (1954); L. F. Larinov, A. S. Khokhlov, E. N. Shkodinskaia, O. S. Vasina, V. I. Trusheikina and M. A. Novikova, *Lancet*, **269**, 169 (1955).

(5) J. M. Luck, *Cancer Res.*, **17**, 1071 (1957); H. E. Smith and J. M. Luck, *J. Org. Chem.*, **23**, 837 (1958).

(6) J. L. Everett, J. J. Roberts and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

(7) H. F. Gram, Carol W. Mosher and B. R. Baker, paper XVIII of this series, THIS JOURNAL, **81**, 3103 (1959).

chloride with methyl hydrocinnamate (I) led to methyl 3-(*p*-acetylphenyl)-propionate (II) in 59% yield. Bromination of II gave methyl 3-(*p*-bromoacetylphenyl)-propionate (III) as an easily crystallizable product in 46% yield. Treatment of III with potassium phthalimide in boiling ethanol afforded the phthalimido derivative IVa in 51% yield. Hydrolysis of IVa gave only a low yield of V, due to the low solubility of IVa in concentrated hydrochloric acid. Hydrolysis in glacial acetic acid–concentrated hydrochloric acid (2:1) gave V in 66% yield. This compound traveled as a single spot in solvent A (R_f 0.13) and in solvent B (R_f 0.59).[8]

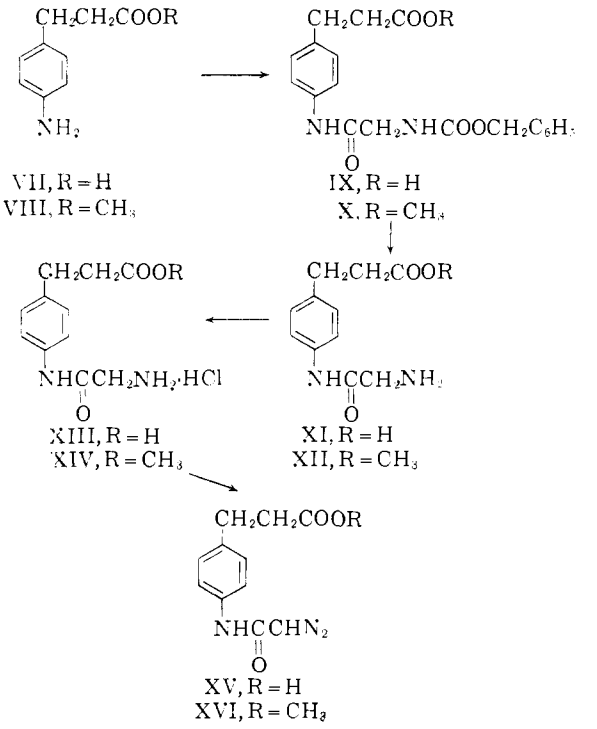


3-(*p*-Glycylphenyl)-propionic acid hydrochloride (V) was diazotized with sodium nitrite in hydrochloric acid at 0-5°. 3-(*p*-Diazoacetylphenyl)-propionic acid (VI) was isolated in 77% yield as a yellow solid, m.p. 210° dec. The stability of the diazoacetyl grouping in such a molecule containing a free carboxylic acid moiety is considered remarkable and the ease of isolation of the compound could be attributed to its insolubility in the reaction media.

Attempts to prepare IVa directly from I in a single step *via* acylation with phthalimidoacetyl chloride resulted in only a 9% yield of IVa.

Treatment of III with sodium azide gave methyl 3-(*p*-azidoacetylphenyl)-propionate (IVb) in 64% yield. Hydrogenation of IVb in dilute hydrochloric acid–ethanol with 5% palladium-on-carbon as catalyst led not only to reduction of the azido group but to reduction of the keto group. This route was accordingly abandoned for the more promising one *via* the phthalimido derivative IVa.

In addition to having a diazoacetyl grouping on phenylpropionic acid as a carrier, it seemed desirable to have another type of aliphatic diazo grouping on the same carrier. The synthesis of 3-(*p*-diazoacetamidophenyl)-propionic acid (XV) from 3-(*p*-aminophenyl)-propionic acid (VII) was therefore undertaken.



3-(*p*-Aminophenyl)-propionic acid (VII) was synthesized in 86% yield by hydrogenation of *p*-nitrocinnamic acid with 5% palladium-on-carbon as catalyst. The sodium salt of VII was condensed with methylcarbonic mixed anhydride of N-carbobenzoxyglycine in aqueous acetone to give 3-[*p*-(N-carbobenzoxyglycylamino)-phenyl]-propionic acid (IX) in yields that varied from 1 to 50% depending on the size of the run. Smaller-scale runs gave higher yields of a purer product. Hydrogenation of IX at 50 p.s.i. (gauge) in 50% aqueous acetic acid with 5% palladium-on-carbon as catalyst removed the carbobenzoxy group to give XI in 94% yield. The melting point, infrared absorption spectrum and analysis varied according to the amount of the acetate salt of XI present when XI was recrystallized from acetic acid–acetone solution.

Compound XI was converted to its hydrochloride XIII in quantitative yield. The hydrochloride traveled as a single spot in solvent B[8] (R_f 0.17), as detected by ultraviolet light or with ninhydrin reagent. All attempts to diazotize XIII using sodium nitrite at low temperatures failed to yield any material showing a diazo band at 4.75 μ in the infrared spectrum.

(8) Paper chromatograms were run by the descending technique on Schleicher and Schuell No. 2043B acetylated paper with benzene–methanol–water (6:2:1) (solvent A), or on Whatman No. 1 paper with water-saturated butanol (solvent B). Spots were detected by observation under ultraviolet light; ninhydrin spray was used for free amino acids.

Since XV appeared to be unstable, it was decided to attempt the synthesis of methyl 3-(*p*-diazoacetamidophenyl)-propionate (XVI). Methyl 3-(*p*-aminophenyl)-propionate (VIII) was prepared in 99% yield by esterification of VII. The condensation of VIII with the methylcarbonic mixed anhydride of N-carbobenzoxyglycine proceeded smoothly, giving a 76% yield of X. This is in contrast to the variable yield obtained from the condensation with the sodium salt of 3-(*p*-aminophenyl)-propionic acid (VII). Removal of the carbobenzoxy group by hydrogenation using 5% palladium-on-carbon in methanol containing one equivalent of hydrochloric acid gave XIV in 60% yield; XIV was also prepared in 50% yield by esterification of XIII.

Diazotization of XIV in dilute hydrochloric acid using sodium nitrite gave XVI in 74% yield as a yellow crystalline solid. All attempts to hydrolyze XVI to yield XV have resulted in loss of the diazo grouping as determined by infrared analysis of the products. Hydrolysis of XVI in one equivalent of sodium hydroxide at 5-10° was not accomplished in 24 hours. The further addition of base caused the disappearance of the ester, diazo and anilide bands in the infrared. Saponification using 1 *N* sodium hydroxide at 5-10° was complete in 45 minutes, but attempted isolation of the diazo acid XV as its barium salt was unsuccessful.

Experimental

Methyl 3-(*p*-Acetylphenyl)-propionate (II).—A three-necked flask was equipped with a mechanical stirrer and a reflux condenser protected from moisture with a calcium chloride drying tube. The apparatus was flushed with nitrogen gas and 29 g. (0.22 mole) of anhydrous aluminum chloride and 60 ml. of Skellysolve B were placed in the flask. To the stirred mixture was added 16.4 g. (0.1 mole) of methyl hydrocinnamate and 7.85 g. (0.1 mole) of acetyl chloride. The reaction mixture was refluxed 4 hours under a stream of nitrogen gas, then poured cautiously into 600 ml. of ice-cold 2 *N* hydrochloric acid, then extracted with methylene chloride (once with 100-ml. followed by three times with 50-ml. portions). The combined organic extracts were washed twice with dilute hydrochloric acid (50 ml.), then once with water (50 ml.), then once with 5% aqueous sodium hydrogen carbonate (25 ml.), and finally twice with water (50 ml.). The organic extract was then dried over anhydrous magnesium sulfate, then the methylene chloride was removed *in vacuo* and the product distilled under vacuum. A mixture (7.03 g.) of methyl hydrocinnamate (I) and the desired product distilled between 76–140° (1.5 mm.), $n^{25}D$ 1.5138 (methyl hydrocinnamate has $n^{25}D$ 1.5059). Methyl 3-(*p*-acetylphenyl)-propionate (II) distilled at 143–146° (1.5 mm.), $n^{25}D$ 1.5291, yield 7.90 g. (59%); λ_{max}^{film} 3.36, 6.21 (aryl), 5.72 (ester C=O), 5.92 (ketone C=O), 8.44, 9.81 (ester C–O–C), 11.91 μ (*p*-disubstituted phenyl). The compound traveled as a single spot (R_f 0.74) in solvent A[8] on acetylated paper, as detected with ultraviolet light.

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.8; H, 6.84. Found: C, 69.9; H, 7.22.

A large-scale preparation gave 353.9 g. of product (63.8% yield).

Methyl 3-(*p*-Bromoacetylphenyl)-propionate (III).—Methyl 3-(*p*-acetylphenyl)-propionate (II) (5.0 g., 24 mmoles) was dissolved in 20 ml. of chloroform and the mixture cooled in an ice-bath, then 3.60 g. (22 mmoles) of bromine was added dropwise over a 20-minute period to the stirred solution and stirring was continued for one hour total time. The reaction mixture was concentrated *in vacuo* to a sirup that crystallized upon cooling in ice and trituration with 15 ml. of methanol. The precipitate was collected on a filter, washed and dried; yield 2.9 g. (46%), m.p. 71–73°. Work-up of the mother liquors gave no further product.

Two recrystallizations from methanol gave an analytical sample, m.p. 78–79°; λ_{max}^{KBr} 5.78 (ester C=O), 5.92 (ketone C=O), 8.37 (ester C–O–C), 11.95, 12.25 μ (*p*-disubstituted phenyl). The compound traveled as a single spot (R_f 0.64) in solvent A[8] on acetylated paper, as detected by ultraviolet light.

Anal. Calcd. for $C_{12}H_{13}BrO_3$: C, 50.5; H, 4.59; Br, 28.0. Found: C, 50.6; H, 4.73; Br, 28.0.

A larger-scale preparation gave 205.8 g. of product (42.5% yield).

Methyl 3-(*p*-Phthalimidoacetylphenyl)-propionate (IVa). (A).—A three-necked flask was equipped with a mechanical stirrer and a reflux condenser protected from moisture with a calcium chloride drying tube, and the apparatus flushed with nitrogen gas. Anhydrous aluminum chloride (1.6 g., 12 mmoles) was placed in the flask with 3 ml. of tetrachloroethane. Then 0.82 g. (5 mmoles) of methyl hydrocinnamate (I) was added followed by 1.12 g. (5 mmoles) of phthalimidoacetyl chloride.[9] The reaction mixture was stirred and heated at 110° under a nitrogen atmosphere for 2 hours, at which time gas evolution was slight. The brown, viscous mass was poured onto a mixture of 60 g. of ice and excess dilute hydrochloric acid. The yellow mixture was extracted three times with 15-ml. portions of ethyl acetate. The combined organic extracts were washed twice with dilute hydrochloric acid (30 ml.), once with 5% aqueous sodium hydrogen carbonate (15 ml.) and twice with water (30 ml.). The organic layer was then dried over anhydrous magnesium sulfate, filtered and the filtrate concentrated *in vacuo* to give 1.0 g. of brown sirup. Trituration of the sirup with methanol induced crystallization. The solid was recrystallized twice from methanol to yield 0.15 g. (9%) of cream-colored product, m.p. 131–133°.

An analytical sample was obtained after a third recrystallization from methanol with Norit treatment, m.p. 140–142°; λ_{max}^{KBr} 5.61, 5.75 (C=O of phthalyl and ester), 8.36 (ester C–O–C), 12.31 (*p*-disubstituted phenyl), 13.85 μ (phthaloyl).

Anal. Calcd. for $C_{20}H_{17}NO_5$: C, 68.3; H, 4.87; N, 3.98. Found: C, 67.9; H, 5.09; N, 3.91.

(B).—A mixture of 19.2 g. (0.067 mole) of methyl 3-(*p*-bromoacetylphenyl)propionate (III) and 12.6 g. (0.068 mole) of potassium phthalimide in 150 ml. of absolute ethanol was refluxed for 2 hours. The hot solution containing salt was filtered and the filtrate chilled. The product which separated was collected on a filter and washed with 50% ethanol–water; yield 12.0 g. (51%), m.p. 143–145°. This product traveled as a single spot (R_f 0.85) in solvent A,[8] as detected by ultraviolet light. This behavior was identical with that of the analytical sample in preparation A. The infrared absorption spectra of the two products were also identical.

3-(*p*-Glycylphenyl)-propionic Acid Hydrochloride (V).—A mixture of 60 g. (0.17 mole) of methyl 3-(*p*-phthalimidoacetylphenyl)-propionate (IVa), 100 ml. of glacial acetic acid and 50 ml. of concentrated hydrochloric acid was refluxed for 6 hours. After being chilled in ice, the mixture was filtered and the filtrate concentrated *in vacuo* to a white solid. The solid was washed with acetone and collected on a filter; yield 2.7 g. (66%) of product that decomposed above 200°.

A portion of the solid was induced to crystallize from absolute ethanol by the addition of a drop of water and cooling in a Dry Ice–acetone-bath; λ_{max}^{KBr} 3.33 (NH_3^+), 3.80 (acidic OH), 5.92 (C=O of acid and ketone), 6.25, 6.70 (aryl, NH_3^+), 7.13 μ (CO_2H). The compound traveled as a single spot in solvent A[8] (R_f 0.13) and in solvent B[8] (R_f 0.59), as detected by ultraviolet light or with ninhydrin reagent (orange-yellow color).

Anal. Calcd. for $C_{11}H_{13}NO_3 \cdot HCl$: C, 54.2; H, 5.79; N, 5.75. Found: C, 54.2; H, 5.78; N, 5.73.

3-(*p*-Diazoacetylphenyl)-propionic Acid (VI).—3-(*p*-Glycylphenyl)-propionic acid hydrochloride (V) (3.5 g., 14 mmoles) was dissolved in 15 ml. of hot water and the solution filtered. The filtrate was acidified with two drops of concentrated hydrochloric acid, stirred and cooled in an ice-bath to 10°. A solution of 1.66 g. (24 mmoles) of sodium nitrite in 5 ml. of water was added dropwise during a 10-minute period. The reaction mixture was stirred at 0–5° for 1 hour and diluted with an equal volume of water. The pale yellow solid that separated was filtered and washed thoroughly with ice-water; yield 2.35 g. (77%) of product that darkened at 120°, sublimed at 180°, melted at 210°

(9) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **71**, 1856 (1949).

dec. After being dried over phosphorus pentoxide at 0°, a sample was analyzed and had λ_{max}^{Nujol} 4.75 (diazo), 5.82 (C=O of acid and ketone), 11.65, 11.98 μ (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.5; H, 4.62. Found: C, 60.7; H, 5.08.

Methyl 3-(*p*-Azidoacetylphenyl)-propionate (IVb).—To a solution of 2.4 g. (8.4 mmoles) of methyl 3-(*p*-bromoacetylphenyl)-propionate (III), 60 ml. of dioxane and 1.64 g. (25 mmoles) of sodium azide was added 40 ml. of water. The solution was allowed to stand at room temperature for 3 days.[10] The resulting mixture was concentrated *in vacuo* to a yellow solid, which was triturated with water, collected on a filter, washed well with water and recrystallized from 20 ml. of methanol; yield 1.34 g. (64%), m.p. 76–81°. This same yield was obtained when the reaction mixture was warmed at 40° for 18 hours. An analytical sample was obtained after one recrystallization from methanol, m.p. 82–83°; λ_{max}^{KBr} 4.75 (N_3), 5.78 (C=O of ester), 5.89 (C=O of ketone), 6.22 (aryl), 8.50 (ester C–O–C), 11.84, 12.18 μ (*p*-disubstituted phenyl). The compound traveled as a single spot in solvent A[8] on acetylated paper (R_f 0.58), as detected by ultraviolet light.

Anal. Calcd. for $C_{12}H_{13}N_3O_3$: C, 58.3; H, 5.29; N, 16.9. Found: C, 58.4; H, 5.49; N, 17.0.

3-(*p*-Aminophenyl)-propionic Acid (VII).—*p*-Nitrocinnamic acid (25 g., 0.13 mole) was shaken in 100 ml. of methyl Cellosolve with 2.5 g. of 5% palladium-on-carbon catalyst under an initial pressure of 55 p.s.i. (gauge) hydrogen, reduction being complete in 2 hours. The solution was filtered, the catalyst washed with ethyl acetate, and the combined filtrate and washings concentrated *in vacuo* to a solid. Recrystallization by solution in 50 ml. of absolute ethanol and addition of 250 ml. of benzene yielded 16.0 g. of a white solid, m.p. 128–131°. The product had R_f 0.59 (red-violet ninhydrin test) in solvent A[8] and R_f 0.73 (red-violet ninhydrin test) in solvent B. A second crop of 2.5 g. (total 86%), m.p. 127–129°, was obtained from the mother liquors. Miersch[11] reported a melting point of 132° for this substance prepared by the sodium amalgam reduction of *p*-nitrocinnamic acid.

Anal. Calcd. for $C_9H_{11}NO_2$: C, 65.4; H, 6.70; N, 8.48. Found: C, 65.7; H, 6.83; N, 8.43.

3-[*p*-(N-Carbobenzoxyglycylamino)-phenyl]-propionic Acid (IX).—3-(*p*-Aminophenyl)-propionic acid (VII) (1.65 g., 10 mmoles) was suspended in 20 ml. of water and 1.68 g. (20 mmoles) of sodium bicarbonate was added with stirring. The solution was cooled to 1° in an ice-bath. A slurry of 2.09 g. (10 mmoles) of N-carbobenzoxyglycine, 1.01 g. (10 mmoles) of triethylamine and 0.95 g. (10 mmoles) of methyl chloroformate was prepared at 10° in 30 ml. of acetone. The slurry[12] was added to the magnetically-stirred solution of VII and a thick paste formed. The reaction mixture was stirred in ice for 1.5 hours, then diluted with water (50 ml.) and the mixture acidified to *p*H 3 with 6 *N* hydrochloric acid. The sticky precipitate was collected on a filter and washed with water. Recrystallization from 20 ml. of ethanol gave 1.6 g. (45%) of product, m.p. 165–167°.

An analytical sample was prepared from 0.5 g. of this crude product by two recrystallizations from 10 ml. of ethanol; m.p. 168–171°; λ_{max}^{KBr} 3.01, 6.48 (NH), 5.90 (C=O of acid and urethan), 5.96 (C=O of amide), 11.90 (*p*-disubstituted phenyl), 13.50, 14.34 μ (monosubstituted phenyl). The compound traveled as a single spot (R_f 0.77) in solvent A[8] on acetylated and Whatman #1 papers, as detected by ultraviolet light. It also traveled as a single spot (R_f 0.70) in solvent B.

Anal. Calcd. for $C_{19}H_{20}N_2O_5$: C, 64.0; H, 5.66; N, 7.86. Found: C, 64.2; H, 5.78; N, 7.80.

3-(*p*-Glycylaminophenyl)-propionic Acid (XI).—An analytically-pure sample of 3-[*p*-N-carbobenzoxyglycylamino)-phenyl]-propionic acid (IX) (0.50 g., 1.4 mmoles) was suspended in 20 ml. of 50% aqueous acetic acid. The suspension was shaken under 35 p.s.i. (gauge) hydrogen for 1.5 hours with 0.2 g. of 5% palladium-on-carbon catalyst. The solution was filtered and the catalyst washed with 10 ml. of water. The combined filtrate and washings were concentrated *in vacuo* to a white solid; yield 0.30 g. (94%), m.p. 240–243° dec. with darkening beginning at 220°.

The solid was dissolved in 10 ml. of water which contained 3 ml. of 10% acetic acid and was reprecipitated by adjusting the *p*H to 6 with ammonia water. Two such treatments gave the product, m.p. 240–241° dec. with darkening beginning at 220°; λ_{max}^{KBr} 3.05 (NH_2), 3.75, 4.75 (N^+), 5.96 (amide C=O), 6.20 (NH_3^+ and COO^-), 6.45 (amide NH), 7.13 (CO_2^-), 11.90 μ (*p*-disubstituted phenyl). The compound traveled as a single spot (R_f 0.03) in solvent B,[8] as detected by ultraviolet light or with ninhydrin reagent (yellow color); however, analysis indicated it was not quite pure.

Anal. Calcd. for $C_{11}H_{14}N_2O_3$: C, 59.4; H, 6.35; N, 12.6. Found: C, 58.8; H, 6.67; N, 11.8.

Another analytical sample was prepared by three recrystallizations from 50% aqueous acetic acid–acetone (1:1), m.p. 221–223° dec. (darkened at 197°). The sample was dried in a desiccator at atmospheric pressure.

Anal. Calcd. for $C_{11}H_{14}N_2O_3 \cdot C_2H_4O_2$: C, 55.3; H, 6.42. Found: C, 55.0; H, 6.51.

3-(*p*-Glycylaminophenyl)-propionic acid (XI) (3.1 g.) was converted to the hydrochloride by recrystallization from 21 ml. of 4 *N* hydrochloric acid. The compound was obtained in nearly quantitative yield (3.5 g.), m.p. 230–240° dec.

An analtyical sample was prepared by a second recrystallization from dilute hydrochloric acid, m.p. 232–237°. The compound traveled as a single spot (R_f 0.17) in solvent B,[8] as detected by ultraviolet and with ninhydrin reagent (yellow changing to violet).

Anal. Calcd. for $C_{11}H_{14}N_2O_3 \cdot HCl$: C, 51.1; H, 5.84; N, 10.8; Cl, 13.7. Found: C, 51.6; H, 6.04; N, 11.2; Cl, 13.4.

All attempts to convert XIII to XV using sodium nitrite at low temperatures failed.

Methyl 3-[*p*-Aminophenyl]-propionate (VIII).—A solution of 10.0 g. (0.06 mole) of 3-(*p*-aminophenyl)-propionic acid (VII) in 150 ml. of absolute methanol saturated with anhydrous hydrogen chloride gas was refluxed for 1 hour. The solution was concentrated *in vacuo* to a solid which was dissolved in water; the *p*H was adjusted to 7 with concentrated ammonia water. The precipitate which formed was collected on a filter, washed with water and dried; yield 10.7 g. (99%), m.p. 51–52°; λ_{max}^{KBr} 2.95 (NH_2), 5.80 (ester C=O), 8.40 (ester C–O–C), 11.95 μ (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{10}H_{13}NO_2$: C, 67.0; H, 7.31; N, 7.82. Found: C, 67.0; H, 7.36; N, 7.57.

Methyl 3-[*p*-(N-Carbobenzoxyglycylamino)-phenyl]-propionate (X).—To a stirred solution of 4.5 g. (0.025 mole) of methyl 3-(*p*-aminophenyl)-propionate (VIII) in 25 ml. of acetone cooled to 0–5° was added a slurry prepared from 5.25 g. (25 mmoles) of N-carbobenzoxyglycine, 2.55 g. (25 mmoles) of triethylamine and 2.35 g. (25 mmoles) of methyl chloroformate in 75 ml. of acetone at 10° in an ice-bath.[12] The suspension was stirred for 1 hour at 0–5°, then diluted with 150 ml. of water. The precipitate was collected on a filter, washed with water and dried; yield 7.10 g. (76%), m.p. 146–149°.

Two recrystallizations of 0.3 g. of crude material from 5 ml. of methanol furnished an analytical sample, m.p. 155–156°; λ_{max}^{KBr} 3.04 (NH), 5.78 (ester C=O), 5.92 (C=O of urethan, amide), 13.40, 14.35 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{20}H_{22}N_2O_5$: C, 64.8; H, 5.98; N, 7.56. Found: C, 64.6; H, 6.08; N, 7.69.

Methyl 3-(*p*-Glycylaminophenyl)-propionate Hydrochloride (XIV). (A).—3-(*p*-Glycylaminophenyl)-propionic acid (XI) hydrochloride (2.0 g., 8 mmoles) was esterified by refluxing in 50 ml. of methanol containing a little anhydrous hydrogen chloride. After being refluxed for 1 hour, the reaction mixture was concentrated *in vacuo*, furnishing 1.1 g. (50% yield) of shiny white platelets, m.p. 198–214°.

A 0.2-g. sample was recrystallized twice from 1 ml. of saturated methanolic hydrogen chloride. The analytical sample so obtained was hygroscopic, m.p. 212–215°, and could not be obtained solvent-free by the usual techniques;

(10) E. D. Nicolaides, R. D. Westland and E. L. Wittle, THIS JOURNAL, **76**, 2887 (1954).

(11) W. Miersch, *Ber.*, **25**, 2109 (1892).

(12) J. R. Vaughan, Jr., and R. L. Osato, THIS JOURNAL, **73**, 5553 (1951); R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

λ_{max}^{Nujol} 5.75 (ester C=O), 5.94 (amide C=O), 6.43 (amide NH, aryl, NH_3^+), 8.57 (ester C–O–C), 12.15 μ (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{12}H_{16}N_2O_3 \cdot HCl$: C, 53.6; H, 6.37. Found: C, 53.0; H, 6.49.

(B).—Methyl 3-[*p*-(N-carbobenzoxyglycylamino)-phenyl]-propionate (X) (7.1 g., 0.019 mole) was suspended with 1.1 g. of 5% palladium-on-carbon catalyst in 50 ml. of methanol containing 0.64 ml. (0.02 mole) of concentrated hydrochloric acid. The suspension was hydrogenated at 50 p.s.i. (gauge) initial pressure for 2 hours, diluted with 50 ml. of methanol and filtered. Concentration of the filtrate *in vacuo* to appearance of crystals gave a shiny white solid which was washed with acetone and collected on a filter; yield 3.30 g. (60%), m.p. 198–208°. The infrared absorption spectrum was identical with that of preparation A.

Methyl 3-(*p*-Diazoacetamidophenyl)-propionate (XVI).—Methyl 3-(*p*-glycylaminophenyl)-propionate hydrochloride (XIV) (6.4 g., 0.022 mole) was dissolved in 25 ml. of water and cooled to 10° in an ice-bath, then 0.5 ml. of concentrated hydrochloric acid was added. A solution of 2.07 g. (0.03 mole) of sodium nitrite in 4 ml. of water was chilled and added dropwise to the acid solution. The yellow product began to precipitate when nitrite addition was nearly complete. After standing 15 minutes at 10–15°, the reaction mixture was filtered and the product washed well with water; yield 4.0 g. (74%), m.p. 110° dec.

An analytical sample was prepared by solution of the product in 95% ethanol at room temperature followed by precipitation with water. After two recrystallizations, the melting point was raised to 118° dec.; λ_{max}^{KBr} 3.10 (NH), 4.82 (diazo), 5.79 (ester C=O), 6.17 (amide C=O), 8.40, 8.58 (ester –C–O–C), 11.85 μ (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{12}H_{13}N_3O_3$: C, 58.83; H, 5.29; N, 17.0. Found: C, 58.3; H, 5.26; N, 16.8.

Attempted saponification of XVI to XV under a variety of conditions was unsuccessful.

ADDED IN PROOF.—The two diazo compounds (VI and XVI) were evaluated[13] against Sarcoma 180, Carcinoma 755, Leukemia L-1210 and Ehrlich ascites. The only positive activity observed was a 37% life extension by VI on mice bearing Ehrlich ascites.

Acknowledgments.—The authors wish to thank Dr. Peter Lim for interpretation of the infrared absorption spectra and his staff for the paper chromatography and spectrophotometry. The authors are also indebted to Mr. O. P. Crews, Jr., and his staff for large-scale preparation of certain intermediates.

(13) We wish to thank Dr. Joseph Greenberg and associates of this Institute for these tests, performed under contract with the Cancer Chemotherapy National Service Center.

MENLO PARK, CALIF.

[CONTRIBUTION FROM THE SCHOOL OF PHARMACY, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO]

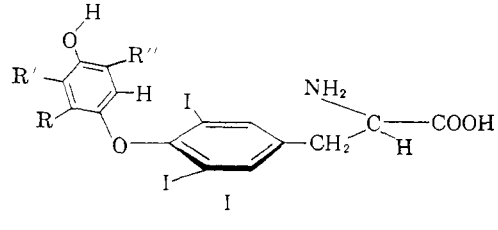
Thyroxine Analogs. I. Synthesis of 3,5-Diiodo-4-(2′-alkylphenoxy)-DL-phenylalanines[1]

BY NICOLAS ZENKER[2] AND EUGENE C. JORGENSEN

RECEIVED MARCH 10, 1959

3,5-Diiodo-4-(2′-methylphenoxy)-DL-phenylalanine and 3,5-diiodo-4-(2′-isopropylphenoxy)-DL-phenylalanine derivatives have been prepared for testing as analogs of thyroxine.

Many analogs of thyroxine have been synthesized in attempts to define structural requirements for thyroid hormonal activity, to elicit selective physiological responses, and to prepare substances capable of antagonizing thyroid hormones.[3] One factor which appears to have received no consideration is the potential difference in orientation for groups occupying the 3′- and 5′-positions of 3,5-diiodothyronine and related diphenyl ethers (I). Molecular models of thyroxine (I, R = H, R′ = B″ = I) indicate a favored perpendicular orientation for the planes of the two phenyl rings, thus



providing a minimal interaction between the bulky 3,5-iodines and the 2′,6′-hydrogens. This preferred orientation may be fixed by placing a bulky group in the 2′-position. This 2′-substituent would be forced to orient away from (distally to) the alanine-bearing ring, but would not be likely to distort the normal diphenyl ether bond angle. A series of such 3,5-diiodo-4-(2′-alkylphenoxy)-DL-phenylalanines was prepared, carrying additional substituents *ortho*, *meta* and *para* to the orienting 2′-group. In this way, the position in space relative to the diiodophenylalanine ring was known for each substituent.

Compounds such as 3,5-diiodo-3′,5′-dimethyl-L-thyronine[4,5] and its thyropropionic acid analog[6,7] have demonstrated the ability of alkyl groups to replace the 3′,5′-iodines of thyroxine analogs with retention of thyroxine-like activity. Thus, oriented alkylthyronines were themselves of biological interest, in addition to their anticipated use in directing iodine substitution into known spatial positions of the phenolic ring. Analogs containing oriented alkyl groups but lacking the 4′-hydroxyl group were prepared to provide further informa-

(1) Reported in part before the Division of Medicinal Chemistry at the 134th Meeting of the American Chemical Society, Chicago, Ill., September, 1958.

(2) In partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California, September, 1958.

(3) H. A. Selenkow and S. P. Asper, Jr., *Physiol. Rev.*, **35**, 426 (1955).

(4) T. C. Bruice, N. Kharasch and R. J. Winzler, *J. Org. Chem.*, **18**, 83 (1953).

(5) T. C. Bruice, R. J. Winzler and N. Kharasch, *J. Biol. Chem.*, **210**, 1 (1954).

(6) N. Kharasch, S. H. Kalfayan and J. D. Arterberry, *J. Org. Chem.*, **21**, 925 (1956).

(7) N. R. Stasilli, R. L. Kroc and R. I. Meltzer, *Endocrinol.*, **64**, 62 (1959).