comparison to AChR ($K_D = 8 \times 10^{-9}$ and $6.8 \times 10^{-8} M$). On the other hand, ACBM strongly binds TEA and procaine whereas these compounds at $10^{-4} M$ have no effect on binding of acetylcholine to AChR.§ Such differences imply that the interaction studied in this communication is indicative of a greater specificity than simple electrostatic attractions. In addition, it has recently been shown# that the affinity of the ACBM for nicotine is very dependent upon the ionic composition of the solution; the affinity increases as the concentration of NaCl is increased up to 2 *M*. This is the opposite of the effect one would expect if electrostatic attractions are the only determinants of the energies of interactions.

In conclusion, the evidence still indicates that the ACBM is on the internal surface of the axon plasma membrane and may be a component of both the Na^+ and K^+ gates. The independence of these two gates is shown pharmacologically by their differential responses to TTX and TEA. However, the interactions with local anesthetics still remain the strongest evidence that the ACBM may be a component common to both.

References

(1) B. Hille, *Progr. Biophys. Mol. Biol.*, **21**, 1 (1970).
(2) R. D. O'Brien, M. E. Eldefrawi, and A. T. Eldefrawi, *Annu. Rev. Pharmacol.*, **12**, 19 (1972).
(3) F. Matsumura and T. Narahashi, *Biochem. Pharmacol.*, **20**, 825 (1971).
(4) (a) J. L. Denburg, M. E. Eldefrawi, and R. D. O'Brien, *Proc. Nat. Acad. Sci. U. S.*, **69**, 177 (1972); (b) J. L. Denburg, *Biochem. Biophys. Acta*, **282**, 453 (1972).
(5) D. Nachmansohn, "Chemical and Molecular Basis of Nerve Activity," Academic Press, New York, N. Y., 1959.
(6) M. E. Eldefrawi, A. T. Eldefrawi, and R. D. O'Brien, *Mol. Pharmacol.*, **7**, 104 (1971).
(7) E. S. Baginski, P. P. Foa, and B. Zak, *Clin. Chim. Acta*, **15**, 155 (1967).
(8) T. Narahashi, J. W. Moore, and W. R. Scott, *J. Gen. Physiol.*, **47**, 965 (1964).
(9) T. Narahashi, N. C. Anderson, and J. W. Moore, *ibid.*, **50**, 1413 (1967).
(10) I. Tasaki and S. Hagiwara, *ibid.*, **40**, 859 (1957).
(11) C. M. Armstrong, *ibid.*, **50**, 491 (1966).
(12) B. Hille, *ibid.*, **51**, 199 (1968).
(13) T. Narahashi and H. G. Haas, *ibid.*, **51**, 177 (1968).
(14) J. F. Hoffman, *ibid.*, **54**, 343 (1969).
(15) J. C. Ellory and R. D. Keynes, *Nature (London)*, **221**, 776 (1969).
(16) P. F. Baker and J. S. Willis, *Biochim. Biophys. Acta*, **183**, 646 (1969).
(17) D. Landowne and J. M. Ritchie, *J. Physiol.*, **207**, 529 (1970).
(18) R. W. Albers, G. J. Koval, and G. J. Siegel, *Mol. Pharmacol.*, **4**, 324 (1968).
(19) T. Narahashi and D. T. Frazier, *Neurosci. Res.*, **4**, 1 (1971).
(20) D. T. Frazier, T. Narahashi, and J. W. Moore, *Science*, **163**, 820 (1969).
(21) P. Krupp, C. P. Bianchi, and G. Suarez-Kurtz, *J. Pharm. Pharmacol.*, **21**, 763 (1969).
(22) T. Narahashi, D. T. Frazier, T. Deguchi, C. A. Cleaves, and M. C. Ernau, *J. Pharmacol. Exp. Ther.*, **177**, 25 (1971).
(23) F. Iverson, *Mol. Pharmacol.*, **7**, 129 (1971).

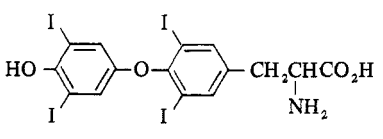
§M. Eldefrawi, unpublished results.
#J. S. Denburg, submitted for publication.

Synthesis of Methylene- and Carbonyl-Bridged Analogs of Iodothyronines and Iodothyroacetic Acids

Spencer L. Tripp,* Fred B. Block, and George Barile
Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Ardsley, New York 10502. *Received April 18, 1972*

Syntheses are described of the methylene- and carbonyl-bridged analogs of a number of iodinated thyronines and thyroacetic acids. The critical synthetic step is arylation of 4-methoxybenzylidenetriphenylphosphorane with methyl 3,5-dinitro-4-chlorobenzoate. An ylide **11** is obtained which decomposes in methanol to yield methyl 3-(4-methoxyphenyl)-4-nitro-2,1-benzisoxazole-6-carboxylate (**13**). Selective reductions of **13** give methyl 3,5-diamino-4-(4-methoxybenzoyl)benzoate (**17**) and methyl 3,5-diamino-4-(4-methoxybenzyl)benzoate (**19**). These diamines are elaborated to the corresponding carbonyl- and methylene-bridged iodothyronines and iodothyroacetic acids.

Since the first synthesis of thyroxine[1] almost half a century ago, an impressive amount of effort has been directed toward the preparation of analogs of this deceptively simple structure (**1**). Much of such work has been stimulated by



1

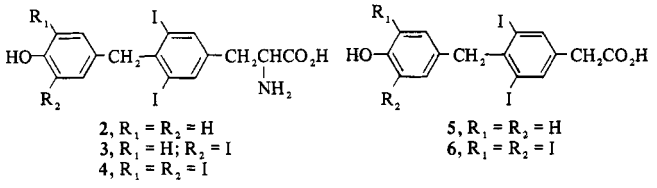
the observations that thyroxine-like activity can be retained, or even increased over that of the natural substance, by appropriate modification.[2] Phenolic-ring iodines can be replaced by alkyl groups, or the outer ring itself by 1-naphthyl.[3] In some instances, inner ring iodines can also be replaced by alkyl.[4] The alanine side chain can also be altered.[5]

A number of intriguing explanations have been advanced for the molecular action of thyroxine. It is known that thyroxine has a rigid, angulated structure, the phenolic ring being perpendicular and inclined at an angle of about 120° to the inner ring. There is also ample evidence to suggest that the outer ring of thyroxine undergoes oxidation, and that analogs which cannot be easily oxidized will not exhibit thyromimetic activity.[2,3a]

Inspection of space-filling models shows that, owing to the steric constraints imposed by the bulky iodine substituents, replacement of the diaryl ether linkage of thyroxine with CO or CH_2 changes the angulated structure very little. On the other hand, methylene substitution would be expected to increase the oxidation potential of the natural hormone considerably.[6] Carbonyl-bridged thyroxine would be even less readily oxidized. Thus, biological testing of these compounds should provide insights into the steric *vs.* electronic basis for the molecular action of thyroxine.

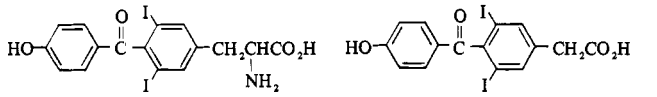
The replacement of the ether function of iodinated thyronines has received relatively little attention. Sulfur-,[7]

CH_2O-,[8] and certain tertiary nitrogen-bridged compounds have been reported.[9] Of these, the diaryl sulfide had approximately 20% of the activity in accelerating tadpole metamorphosis. We now wish to report the methylene-bridged analogs (**2–6**) of DL-thyroxine, tetraiodothyroacetic acid, and related species and the carbonyl-bridged analogs (**7**, **8**) of 3,5-diiodo-DL-thyronine and 3,5-diiodothyroacetic acid.



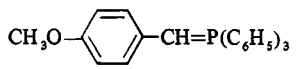
2, $R_1 = R_2 = H$
3, $R_1 = H$; $R_2 = I$
4, $R_1 = R_2 = I$

5, $R_1 = R_2 = H$
6, $R_1 = R_2 = I$



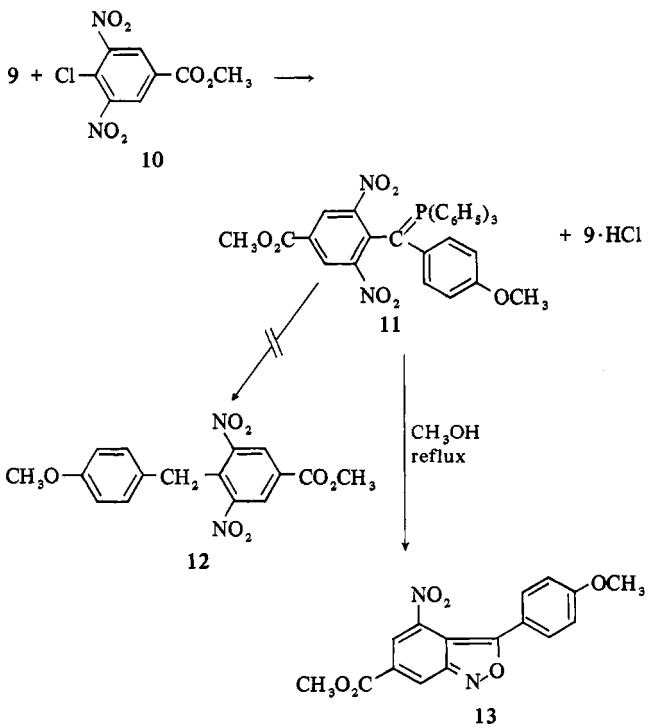
7 8

The diaryl ether linkage of synthetic thyroxines is generally formed by nucleophilic attack of a phenolate anion on an activated aromatic substrate.[10] Using this reaction as a model, we wished to perform a similar displacement with a carbon nucleophile. 4-Methoxybenzylidenetriphenylphosphorane (**9**) was chosen for this purpose. Pappas and



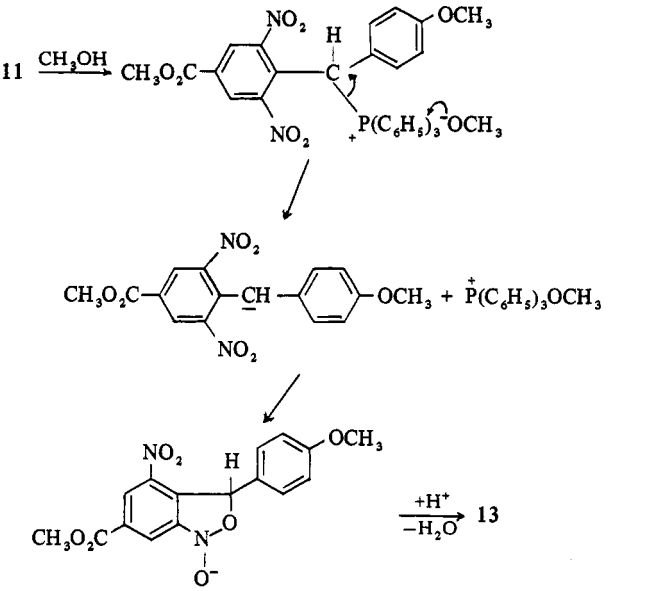
9

Gancher[11] have reported the arylation of various ylides with 2,4-dinitrochlorobenzene and picryl chloride. It was hoped that methyl 3,5-dinitro-4-chlorobenzoate (**10**) would thus react with 2 equiv of **9** to form ylide **11**, which could then be decomposed in methanol to yield the diarylmethane **12**.

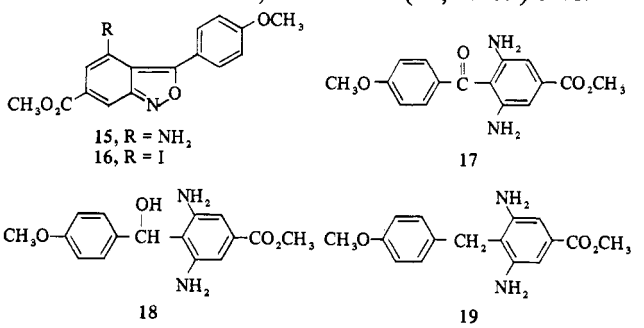


10 11 12 13

When the reaction was run in benzene at room temperature, **11** was indeed obtained, but decomposition in refluxing methanol gave methyl 3-(4-methoxyphenyl)-4-nitro-2,1-benzisoxazole-6-carboxylate (**13**) as a major product; no **12** could be isolated. The reaction probably proceeds by methanolysis of ylide **11**, followed by cyclization of the resulting benzyl anion.[12]



Isolation of benzisoxazole **13** proved fortuitous, as a route was now available to carbonyl-bridged thyroxines as well as the methylene-bridged compounds. Reduction of benzisoxazoles to *o*-aminobenzophenones has been extensively investigated,[13] as the latter compounds are precursors to benzodiazepines. Korte and Behner[14] have reported that more stringent conditions will lead to *o*-aminobenzhydrols and *o*-aminodiarylmethanes. By modifying the literature conditions, four amines (**15**, **17–19**) could



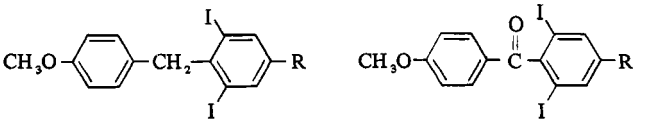
15, R = NH_2
16, R = I

17

18 19

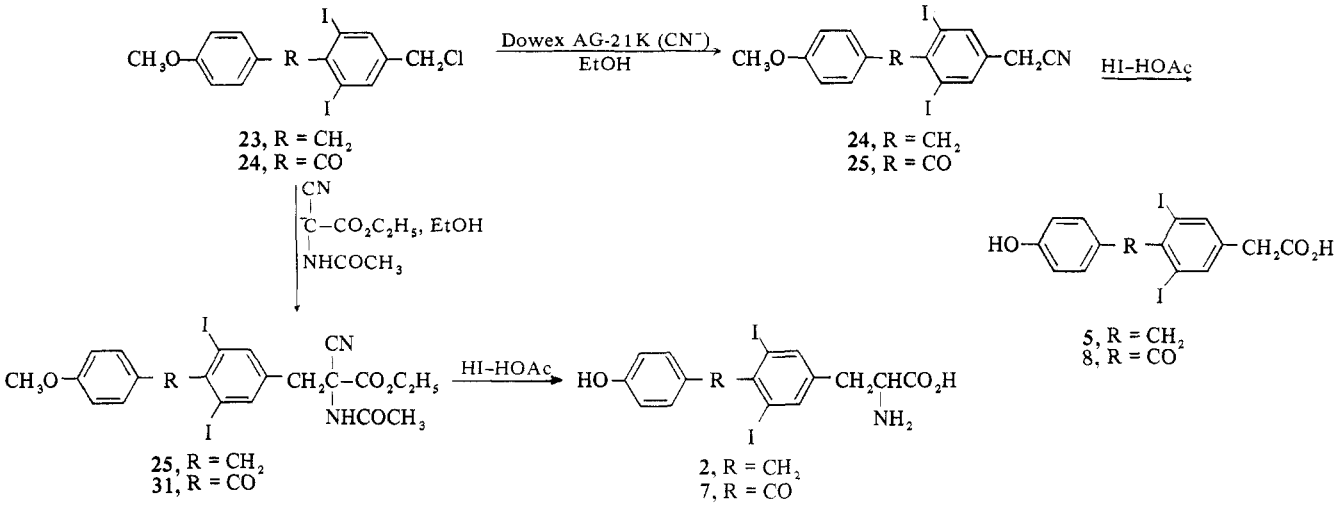
be prepared from **13**. Although catalytic reduction apparently occurs stepwise, 4-aminobenzisoxazole (**15**) could never be obtained free from the next reduction product (**17**). It was isolated as an unstable solid by zinc–acetic acid reduction of **13**. Characterization is based on conversion to the 4-iodo analog **16** by diazotization–iodination.

Benzophenone **17** and diarylmethane **19** were prepared from benzisoxazole **13** by hydrogenation in 5% acetic acid–ethyl acetate (PtO_2, 1 atm, 25°, 24 hr) and methanol (Raney nickel, 500 psi, 100°, 18 hr), respectively. Both **17** and **19** were tetrazotized and iodinated to diiodo analogs **26** and **20**. It was anticipated that acetic acid, propionic



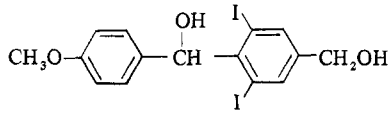
20, R = CO_2CH_3
21, R = CO_2H
22, R = CH_2OH
23, R = CH_2Cl
24, R = CH_2CN
25, R = $CH_2C(CN)CO_2C_2H_5$
$NHCOCH_3$

26, R = CO_2CH_3
27, R = CO_2H
28, R = CH_2OH
29, R = CH_2Cl
30, R = CH_2CN
31, R = $CH_2C(CN)CO_2C_2H_5$
$NHCOCH_3$



acid, or alanine side chains would be most favorable in conferring thyromimetic activity. For this reason a common precursor was desired which could be converted into these functions, particularly the acetic acid and alanine derivatives. We therefore planned to prepare benzyl chlorides **23** and **29** for alkylation with ethyl acetamidocyanoacetate anion or cyanide. In the methylene series, benzyl chloride 23 was prepared from ester **20** by acidic hydrolysis, diborane reduction, and treatment with thionyl chloride. Reaction with the sodium salt of ethyl acetamidocyanoacetate in ethanol and demethylation-hydrolysis in refluxing 1:1 hydriodic acid-acetic acid gave 3,5-diiodo-DL-thyronine analog **2** in a straightforward manner. The phenolic ring of **2** was mono- and diiodinated to **3** and **4**.

To prepare acetic acid analogs, **23** was treated with cyanide-equilibrated anion-exchange resin,[15] hydrolyzed, demethylated to **5**, and diiodinated to **6**. In the carbonyl series, difficulties were encountered with the diborane reduction of acid **27** to keto alcohol **28**. It had been thought possible to reduce this acid without disturbing the highly hindered benzophenone function. Diborane reductions at room temperature, however, invariably gave mixtures of the desired compound and diol **32**. By running the reaction at low temperatures and avoiding large excesses of diborane, it was possible to prepare **28** selectively. Separation of **28** from small amounts of **32** could be accomplished by fractional



32

crystallization from methanol. Later it was found that in the presence of a small amount of sodium borohydride, little or no over-reduced diol was formed, and the reaction could be run at room temperature. We do not have a satisfactory explanation for this unusual observation.

Chlorination of keto alcohol **28**, and the reaction sequence to 3,5-diiodo-DL-thyronine analog **7** and 3,5-diiodothyroacetic acid analog **8**, was carried out as described for the methylene-bridged series, and the yields are comparable. Attempts to iodinate the phenolic ring of **7** and **8** using iodine-potassium iodide in aqueous methylamine or iodine monochloride in acetic acid failed. The compounds were destroyed on refluxing with these reagents, but only a very low degree of iodination was achieved at room temperature. Similar results were obtained with bromine in acetic acid.

Initial biological data† indicate that compounds **2–8** significantly elevate levels of mitochondrial glycerophosphate dehydrogenase in the liver of rats. The most potent analog is **3**, having approximately 5% the activity of 3,3′,5-triiodo-L-thyronine. As in the oxygen-bridged series, methylene-bridged diiodinated compounds are less active than their tri- or tetraiodinated analogs, but the decrease in potency is much less, so that **2** has 34% the activity of 3,5-diiodo-DL-thyronine and **5** shows 18% of the activity of 3,5-diiodothyroacetic acid. A similar comparison with the carbonyl-bridged compounds **7** and **8** gives values of 13 and 9%, respectively. Oxygen consumption of rats given compound **3** orally at a dosage of 0.2 μmole/100 g was 35% higher than that of the controls. Compound **4** similarly elevated metabolic rate in the thyroidectomized animals, so the action of **4** is not mediated by endogenous thyroid hormones.

In conclusion it appears that an oxygen or sulfur bridge in iodothyronines is not necessary for thyromimetic activity. The weaker activity of the methylene- and carbonyl-bridged compounds is probably due to their higher oxidation potential, but the fact that some activity is retained emphasizes the importance of steric considerations.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra (as Nujol mulls) and nuclear magnetic resonance spectra were obtained on a Perkin-Elmer Model 137 spectrophotometer and a Varian A-60A spectrometer (Me_4Si), respectively. Thin-layer chromatograms were prepared using Brinkmann silica gel F-254 plates. Water content of hydrated compounds was confirmed by Karl Fischer titration. Methyl 3,5-dinitro-4-chlorobenzoate[17] and 4-methoxybenzyltriphenylphosphonium chloride[18] were prepared by literature methods. Commercial diborane in THF (Alfa) was standardized by careful decomposition of aliquots with wet Et_2O, solvent removal, and titration of the residual boric acid with NaOH. Phenolphthalein was used as an indicator, and mannitol was added during the titration. All reactions were run under nitrogen. The following solvents were stored over drying agents and used directly: benzene, THF (calcium hydride), and ethanol (molecular sieve 4A).

Methyl 3-(4-Methoxyphenyl)-4-nitro-2,1-benzisoxazole-6-carboxylate (13). 4-Methoxybenzyltriphenylphosphonium chloride (83.78 g, 0.20 mole) was suspended in 800 ml of PhH and treated with 0.20 mole of *n*-butyllithium in hexane. After the reaction had been stirred for 1 hr, 26.60 g (0.1 mole) of methyl 3,5-dinitro-4-chlorobenzoate in 300 ml of PhH was added. The solution, initially dark orange, rapidly turned purplish black and was stirred overnight

†We thank Dr. S. Psychoyos for providing the biological data. Assays of liver mitochondrial glycerophosphate dehydrogenase levels are based on the work of Richert, *et al.*[16] A detailed report will be published elsewhere.

and filtered. Washing with warm PhH removed most of the color from the filter cake. Filtrate and washings were combined and evaporated to give 62.0 g of crude ylid **11** as a dark semisolid. This material was taken up in 900 ml of MeOH and refluxed for 1 hr, during which time the initial purple solution became yellow and deposited a precipitate. Chilling and filtration afforded 17.22 g of yellow needles. Two recrystallizations from PhH–MeOH gave 15.50 g (47%) of **13**: mp 199–200°; nmr (DMSO-d_6) δ 4.10 (s, 3, CH_3), 3.90 (s, 3, CH_3), 7.09–7.68 (A_2B_2, 4, aromatic), 8.35 (s, 1, aromatic), 8.68 (s, 1, aromatic); ir 1730 cm^{-1} (ester C=O). *Anal.* ($C_{16}H_{12}N_2O_6$) C, H, N.

A sample of crude ylide **11** was purified by Soxhlet extraction with Et_2O, evaporation of the Et_2O and two recrystallizations from $CHCl_3$ (EtOH-free)–hexane. Pure **11**, mp 128.5–130° dec, is an air stable, dark purple solid, readily soluble in most organic solvents to give reddish purple solutions. *Anal.* ($C_{34}H_{27}N_2O_7P$) C, H, N, P.

Methyl 3-(4-Methoxyphenyl)-4-iodo-2,1-benzisoxazole-6-carboxylate (16). To a solution of 6.00 g (18.3 mmoles) of **13** in 700 ml of $CHCl_3$ was added 5.10 g (78.0 g-atoms) of Zn and 10 ml of AcOH. The reaction mixture was refluxed 3 hr, filtered, washed with 2 *N* NaOH and H_2O, dried (Na_2SO_4), and evaporated to dryness *in vacuo*. Trituration with MeOH left a white solid, which was recrystallized from MeOH to afford 1.90 g (35%) of methyl 3-(4-methoxyphenyl)-4-amino-2,1-benzisoxazole-6-carboxylate (**15**).

Previous experience with **15** indicated that it was unstable on standing, so the 4-iodo analog **16** was prepared immediately. A solution of nitrosylsulfuric acid was prepared by the addition of 440 mg (6.39 mmoles) of $NaNO_2$ to 15 ml of H_2SO_4 kept at 40°. After the addition, the solution was cooled, 15 ml of AcOH added, and the the whole added dropwise to a solution of 1.90 g (6.39 mmoles) of **15** in 60 ml of H_2SO_4 and 30 ml of AcOH, kept at 0–2°. The reaction mixture was stirred for an additional 45 min at this temperature, then poured into a rapidly stirred, chilled mixture of 4.0 g of I_2, 7.0 g of KI, 1.5 g of urea, 120 ml of H_2O, and 60 ml of $CHCl_3$. Following overnight stirring at room temperature, the $CHCl_3$ layer was separated and the aqueous portion extracted twice with 60-ml portions of $CHCl_3$. The combined organic phases were washed with equal volumes of H_2O, 10% aqueous sodium bisulfite (twice), and H_2O. Drying (Na_2SO_4) and removal of solvent left 1.21 g (46%) of **16**. An analytical sample was prepared by recrystallization from EtOAc, mp 170–172°. *Anal.* ($C_{16}H_{12}INO_4$) C, H, I, N.

Methyl 3,5-Diamino-4-(4-methoxybenzoyl)benzoate (17). Benzisoxazole (**13**), 15.0 g (45.7 mmoles), was hydrogenated at atmospheric pressure in 800 ml of EtOAc containing 40 ml of AcOH and 1 g of PtO_2. Initial warming was necessary to ensure solution of **13**. After 24 hr, catalyst was removed by filtration, and the orange solution taken to dryness under reduced pressure. Trituration with 2:1 hexane–EtOAc, followed by recrystallization from EtOAc–hexane, afforded 10.59 g (76%) of yellow crystals, mp 130–131°. *Anal.* ($C_{16}H_{16}N_2O_4$) C, H, N.

Methyl 3,5-Diamino-4-(4-methoxy-α-hydroxybenzyl)benzoate (18). Benzisoxazole (**13**), 1.00 g (3.05 mmoles), was suspended in 150 ml of MeOH and hydrogenated in the presence of Raney nickel (W. R. Grace, No. 28) for 2 hr at 60° and 50 psi. The solution was filtered, evaporated to dryness under reduced pressure, and taken up in a warm mixture of PhH–AcOH (9:1). The product, 210 mg (23%), precipitated. An analytical sample was obtained by recrystallization from MeOH, mp 187–188°. *Anal.* ($C_{16}H_{18}N_2O_4$) C, H, N.

Methyl 3,5-Diiodo-4-(4-methoxybenzyl)benzoate (20). Benzisoxazole (**13**), 6.56 g (20 mmoles), was suspended in 350 ml of MeOH and a teaspoon of Raney nickel was added. Following hydrogenation (100°, 500 psi) for 18 hr, the colorless solution was filtered, and the solvent was removed under reduced pressure. The residue was recrystallized from EtOAc–hexane to yield 3.12 g (55%) of diamine **19**, mp 133–134°. This diamine could not be obtained analytically pure and was converted to the diiodo analog **20** by the method described for 4-iodobenzisoxazole **16**. Thus, 5.60 g (19.6 mmoles) of diamine **19** was tetrazotized with 39.2 mmoles of nitrosylsulfuric acid. Iodination and work-up by the previously described procedure gave 9.86 g of a dark oil, which was chromatographed on silica gel using $CHCl_3$ as an eluent, yield of **20**, 4.86 g (49%). An analytical sample was obtained by recrystallization from MeOH, mp 125.5–126°. *Anal.* ($C_{16}H_{14}I_2O_3$) C, H, I.

Methyl 3,5-Diiodo-4-(4-methoxybenzoyl)benzoate (26). Diamine **17**, 30.03 g (0.10 moles), was tetrazotized with 0.20 mole of nitrosylsulfuric acid and iodinated by the method described for **16**. Work-up gave a solid, which was recrystallized from CH_2Cl_2–MeOH to yield 22.29 g (43%) of diiodo ester **26**. An analytical sample was prepared by one further recrystallization, mp 191.5–192.5°. *Anal.* ($C_{16}H_{12}I_2O_4$) C, H, I.

3,5-Diiodo-4-(4-methoxybenzyl)benzyl Alcohol (22). Diiodo ester **20** (3.72 g, 7.32 mmoles) was refluxed in a mixture of 400 ml of AcOH and 150 ml of 6 *N* HCl for 4 hr. *In vacuo* removal of solvents left 3.32 g (91.5%) of 3,5-diiodo-4-(4-methoxybenzyl)benzoic acid (**21**). The material was nearly homogeneous to tlc (10% AcOH–PhH), was vacuum dried at 120°, and was used without further purification.

Acid **21** (3.32 g, 6.72 mmoles) was dissolved in 300 ml of dry THF, cooled, and treated with 40 ml of 0.45 *M* diborane in THF. After 5-hr reflux, the solution was cooled, ice was added to destroy residual diborane, and volatile materials were removed by distillation under reduced pressure. The solid was washed well with H_2O and vacuum dried at 80°, yield 3.14 g (97%). An analytical sample was obtained by recrystallization from MeOH, mp 147–148°. *Anal.* ($C_{15}H_{14}I_2O_2$) C, H, I.

3,5-Diiodo-4-(4-methoxybenzyl)benzyl Chloride (23). To a solution of 14.28 g (29.75 mmoles) of **22** in 500 ml of dry PhH was added 6 ml of $SOCl_2$ followed by a drop of DMF. The mixture was heated at 40° for 4 hr and let stand overnight. Volatile materials were removed under reduced pressure and the residue, chromatographed on silica gel (toluene eluent), afforded 13.93 g (94%) of benzyl chloride **23**: recrystallized from MeOH, mp 94–95°. *Anal.* ($C_{15}H_{13}ClI_2O$) C, H, Cl, I.

3,5-Diiodo-4-(4-hydroxybenzyl)-DL-phenylalanine Hemihydrate (2). Ethyl cyanoacetamidoacetate (3.02 g, 17.7 mmoles) was added to a solution of Na (407 mg, 17.7 mg-atoms) in 100 ml of EtOH, followed by 2.15 g (4.13 mmoles) of benzyl chloride **23**. The mixture was refluxed 4 hr, solvent removed under reduced pressure, and the residue washed with 5% aqueous $NaHCO_3$ and water. The resulting amorphous white solid was chromatographed on silica gel. A first fraction ($CHCl_3$ eluent) contained 430 mg of unreacted **23**, the second ($CHCl_3$–EtOAc eluent) gave 1.87 g of a colorless oil showing two components by tlc. No attempt was made to separate the components of the second fraction. Instead, 1.73 g was dissolved 100 ml in a 1:1 mixture of AcOH and concd HI. After refluxing 4 hr, volatile materials were distilled *in vacuo*, and H_2O was added. The suspended product was again reduced to dryness, and the procedure repeated twice to remove traces of HI. Warm EtOH, 25 ml, was added to dissolve the residue, and this solution was treated carefully with 10% aqueous sodium acetate to raise the pH to 5. After refrigeration, the free amino acid was collected by filtration and washed with H_2O until the washings showed a negative halide test with $AgNO_3$. Yield of **2** after vacuum drying at 80° was 1.25 g. The product is homogeneous to tlc (ammonia-saturated butanol, 10:10:1 toluene–AcOH–H_2O) and shows ir absorptions (3250–3150, 1620, 1590, 1515 cm^{-1}) characteristic of an amino acid. An analytical sample was prepared by two more isoelectric precipitations and vacuum dried at 80°, mp 222–225° dec. *Anal.* ($C_{16}H_{25}I_2NO_3 \cdot 0.5H_2O$) C, H, I, N.

3,5-Diiodo-4-(3-iodo-4-hydroxybenzyl-DL-phenylalanine Hemihydrate (3). A solution of 1.57 g (2.95 mmoles) of diiodoamino acid **2** in 110 ml of 40% aqueous $MeNH_2$ was cooled to 5°. Iodine (742 mg, 2.92 mequiv) in 20 ml of 10% aqueous KI was added in one portion to the rapidly stirred solution. After 10 min, concd HCl (~100 ml) was added while keeping the solution below 15°. When the pH reached 5, the solution was refrigerated and the precipitate removed by centrifugation. The precipitate was washed well with water and suspended in 50 ml of EtOH, and concd HCl (~0.5 ml) was added to give a slightly cloudy solution. After filtration, the EtOH was warmed, and 30 ml of H_2O added. The hot solution was then neutralized to pH 5 with 10% aqueous sodium acetate and cooled, and the precipitate removed and washed by centrifugation. This precipitation was performed three more times to give, after vacuum drying at 80°, 1.78 g of **3** (91%), mp 204–207° dec. The product was homogeneous to tlc in the systems described for compound **2**. *Anal.* ($C_{16}H_{14}I_2NO_2 \cdot 0.5H_2O$) C, H, I, N.

3,5-Diiodo-4-(3,5-diiodo-4-hydroxybenzyl)-DL-phenylalanine (4). A solution of 1.13 g (2.165 mmoles) of diiodoamino acid **2** was dissolved in 20 ml of 40% aqueous $MeNH_2$ and iodinated with 1.10 g (4.35 mequiv) of I_2 in 10 ml of 10% aqueous KI. The addition of iodine solution was carried out at room temperature over 5 min. After stirring for an additional 35 min, the reaction mixture was chilled and the pH lowered to 5 by slow addition of 6 *N* HCl. The free amino acid, 2.12 g, was collected and reprecipitated three more times by the procedure described for **3**: yield of **4**, 1.39 g (83%); mp 215–216° dec. The product was homogeneous to tlc in the systems described for compound **2**. *Anal.* ($C_{16}H_{13}I_4NO_3$) C, H, I, N.

3,5-Diiodo-4-(4-hydroxybenzyl)phenylacetic Acid (5). Benzyl chloride **23**, 5.12 g (10.3 mmoles), was dissolved in 600 ml of absolute EtOH. The solution was treated with 60 cm^3 of the cyanide form of Dowex AG-21K resin[15] and stirred for 4 hr at 60°. The dark red resin was removed by filtration, washed with EtOH, and discarded. The combined filtrates and washings were concentrated under reduced pressure, and the resulting oil (4.1 g) was chromatographed on silica gel ($CHCl_3$ eluent). A major fraction of 3.75 g of nitrile **24** was obtained as a yellow oil, which crystallized on standing (mp 100–101.5°). Other attempts using a smaller amount of resin were less satisfactory.

Nitrile **24**, 3.75 g (7.57 mmoles), was dissolved in 40 ml of a 1:1 mixture of AcOH and concd HI. After 4-hr reflux and overnight standing, the mixture was filtered, and the white precipitate washed well with water to give 3.31 g of **5** (65% from **23**). An analytical sample was obtained by recrystallization from HOAc–water, mp 207–208°. *Anal.* ($C_{15}H_{12}I_2O_3$) C, H, I.

3,5-Diiodo-4-(3,5-diiodo-4-hydroxybenzyl)phenylacetic Acid (6). Diiodophenylacetic acid **5**, 3.08 g (6.24 mmoles), was dissolved in 300 ml of 40% aqueous $MeNH_2$, cooled to 5°, and iodinated (3.18 g, 12.5 mequiv of I_2 in 150 ml of 10% aqueous KI) according to the procedure for **4**. The reaction mixture was stirred 3 hr, chilled, and acidified with concd HCl. The precipitate was filtered, washed with H_2O, and recrystallized twice from AcOH–H_2O to give 3.57 g of **6** (77%), mp 206–209°. *Anal.* ($C_{15}H_{10}I_4O_3$) C, H, I.

3,5-Diiodo-4-(4-methoxybenzoyl)benzoic Acid (27). Diiodo ester **26** was hydrolyzed as previously described (see preparation of **22**). **26**, 8.21 g (15.7 mmoles), gave 7.99 g of crude acid **27**. This material was recrystallized from MeOH to yield 7.34 g of **27** (92%), mp 124.5–126°. *Anal.* ($C_{15}H_{10}I_2O_4$) C, H, I.

3,5-Diiodo-4-(4-methoxybenzoyl)benzyl Alcohol (28). Method A. A number of runs were made to prepare keto alcohol **28**. The reaction is capricious and must be carefully followed by tlc to ensure success. Large excesses of diborane and temperatures above 0° must be avoided.

To 360 ml of dry THF was added 6.10 g (12 mmoles) of acid **27**. After cooling to −30°, a solution of 0.4 *M* diborane in THF, 28.8 ml (11.5 mmoles), was added. The reaction was transferred to a low temperature bath (kryostat UK-30, Lauda Instruments, Inc.) and run for a total of 45 hr at −10 to 0°. Aliquots for tlc were evaporated to dryness, and the residues partitioned between $CHCl_3$ and H_2O. The dry (Na_2SO_4) $CHCl_3$ layers were spotted on silica gel plates and developed with Et_2O. Visualization was by short-wave uv.

During the course of the reaction, starting acid **27** (R_f 0) diminished and product (R_f 0.35) appeared. A third component, diol **32** (R_f 0.43), also appeared, and the reaction was worked up when the spots for **27** and **32** were of about equal intensity. Generally it was necessary to add small amounts of diborane solution as the reaction progressed. An additional 4.5 ml (1.8 mmoles) was added after 16 hr in this particular run.

After 45 hr, ice was added to destroy residual diborane, and the whole was taken to dryness under reduced pressure. The residue was extracted several times with small portions of 2 *N* NaOH. Acidification of this extract gave 50 mg of acid **27**. The undissolved material was washed with H_2O, dried, and dissolved in 500 ml of warm MeOH. Successive reductions of the volume precipitate first the desired product **28**, 4.98 g (84%), then diol **32**, 300 mg.

An analytical sample of **28** was obtained by recrystallization from MeOH, mp 181.5–183°. *Anal.* ($C_{15}H_{12}I_2O_3$) C, H, I.

An analytical sample of diol **32** was obtained by recrystallization from EtOAc–hexane: mp 141.5–143°; nmr (DMSO-d_6) δ 3.70 (s, 3, CH_3), 4.43 (d, J = 6 Hz, 2, CH_2), 5.28 (t, J = 6 Hz, 1, $ArCH_2OH$), 6.00 (d, J = 5 Hz, 1, Ar_2CHOH), 6.19 (d, J = 5 Hz, 1, Ar_2CH). *Anal.* ($C_{15}H_{14}I_2O_3$) C, H, I.

Method B. To 250 ml of THF was added 3.00 g (5.9 mmoles) of **27**. After cooling to ~−30°, 38.4 ml (15.3 mmoles) of a 0.4 *M* solution of diborane in THF was added, followed by 20 mg of $NaBH_4$. The reaction was allowed to come to room temperature and stirred an additional 4 hr. MeOH was added, and volatile materials were removed *in vacuo*. The residue was taken up in $CHCl_3$ and washed with H_2O, and the organic phase dried over Na_2SO_4. Removal of solvents under reduced pressure gave 2.6 g of **28**, recrystallized from MeOH to yield 2.42 g (82%), mp 181–183°

3,5-Diiodo-4-(4-methoxybenzoyl)benzyl Chloride (29). Keto alcohol **28**, 4.45 g (9.0 mmoles), was chlorinated by the method described for **23**. Removal of solvent from the reaction mixture left an oil, which crystallized on trituration with MeOH, and was recrystallized from this solvent. Yield of **29** was 4.19 g (91%). An analytical sample was obtained by an additional recrystallization, mp 160.5–151.5°. *Anal.* ($C_{15}H_{11}ClI_2O_2$) C, H, I.

3,5-Diiodo-4-(4-hydroxybenzoyl)-DL-phenylalanine Hydrate (7). Benzyl chloride **29**, 2.05 g (4.0 mmoles), was alkylated with ethyl cyanoacetamidoacetate by a previously described procedure (**2**). Work-up gave 2.73 g of a light yellow oil which was chromatographed on silica gel (EtOAc eluent). From the first fraction 50 mg of unreacted starting material was recovered. The second fraction gave 2.11 g of a colorless oil. A third component, 360 mg, was eluted with 10% EtOH–EtOAc. This last material is probably a partially hydrolyzed product of the **29** adduct. Products from fractions two (2.00 g) and three (360 mg) were hydrolyzed separately to give 1.61 g and 288 mg of **7**, respectively. The two crops were combined and purified by two precipitations at pH 5, then vacuum dried at 80°. Yield of **7** was 1.51 g (68% from **29**), mp 220–225°. *Anal.* ($C_{16}H_{13}I_2NO_4 \cdot 0.8H_2O$) C, H, I, N.

3,5-Diiodo-4-(4-hydroxybenzoyl)phenylacetic Acid (8). Benzyl chloride **29**, 2.97 g (5.8 mmoles), was treated with the cyanide form of Dowex AG-21K resin[15] as described previously (**5**). An analogous work-up gave 1.80 g of nitrile **30** as a colorless oil. This material was hydrolyzed and demethylated in 20 ml of a 1:1 mixture of AcOH and concd HI. After 4-hr reflux, the solution was concentrated *in vacuo*, and a white solid precipitated. The solid was removed by filtration, washed well with H_2O, and recrystallized twice from AcOH–H_2O to give 1.30 g of **8** (67%), mp >250°. *Anal.* ($C_{15}H_{10}I_2O_4$) C, H, I.

Acknowledgments. We thank Mr. Ernst Bachmann, Mr. Lawrence DellaVecchia, and Mr. Arthur Schroder for excellent technical assistance; and the Analytical Research Department for analyses and spectral data.

References

(1) C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).
(2) E. C. Jorgensen, *Mayo Clin. Proc.*, 39, 560 (1964).
(3) (a) S. B. Barker, *Gumma Symp. Endocrinol., Proc.*, **3**, 181 (1965); (b) E. C. Jorgensen, P. A. Lehman, C. Greenberg, and N. Zenker, *J. Biol. Chem.*, **237**, 3832 (1962).
(4) R. E. Taylor, Jr., T. Tu, S. B. Barker, and E. C. Jorgensen, *Endocrinology*, **80**, 1143 (1967).
(5) A. H. Selenkow and S. P. Asper, Jr., *Physiol. Rev.*, **35**, 426 (1955).
(6) (a) H. R. Gersmann and A. F. Bickel, *J. Chem. Soc.*, 2356 (1962); (b) J. Wynn and W. Fore, *J. Biol. Chem.*, **240**, 1766 (1965); (c) J. Wynn and R. Gibbs, *ibid.*, **238**, 3490 (1963).
(7) C. R. Harington, *Biochem. J.*, **43**, 434 (1948).
(8) J. H. Barnes, J. Elks, F. F. Stephens, and G. J. Waller, *J. Chem. Soc.*, 764 (1953).
(9) (a) R. C. Cookson, *ibid.*, 643 (1953); (b) R. Mukherjee and P. Block, Jr., *J. Chem. Soc. C*, 1596 (1971).
(10) J. H. Wilkinson, *Biochem. J.*, **63**, 601 (1956).
(11) J. J. Pappas and E. Gancher, *J. Org. Chem.*, **31**, 1287 (1966).
(12) J. D. Loudon and G. Tennant, *Quart. Rev., Chem. Soc.*, 18, 394 (1964).
(13) (a) G. N. Walker, *J. Org. Chem.*, 27, 1929 (1962); (b) F. Hoffmann-LaRoche and Co., A.-G., Netherlands Application 6,407,011; *Chem. Abstr.*, **63**, 583 (1965).
(14) F. Korte and O. Behner, *Justus Liebigs Ann. Chem.*, **621**, 51 (1959).
(15) M. Gordon, M. L. DePamphilis, and C. E. Griffin, *J. Org. Chem.*, **28**, 698 (1963).
(16) D. A. Richert, J. Schenkman, and W. W. Westerfeld, *J. Nutr.*, **83**, 332 (1964).
(17) J. Miller, *J. Amer. Chem. Soc.*, 77, 180 (1955).
(18) R. Ketcham, D. Jambotkar, and L. Martinelli, *J. Org. Chem.*, 27, 4666 (1962).