2-Benzyl- and 2-Phenyl-3,4-dihydroxyphenylalanine[1]

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A large number and variety of compounds prolong the survival time of chick embryos infected with certain viruses, or delay the appearance of cytopathic changes caused by viruses growing in tissue cultures.[2] Among these are DL-β-(2-fluoro-) and especially DL-β-(2-methyl-3,4-dihydroxyphenyl)alanine[3] which inhibit the PR-8 strain of the influenza virus in the chick embryo. However, as with so many other "*in vitro–in vivo*" active materials, this activity was not maintained when the amino acids were tested on mice or rats infected with the same virus. There is no evidence linking this failure with transport difficulties or with the presence of antimetabolites in mammals. In this dilemma our attention was drawn to observations by Cavallini and Massarani,[4] who had to deal with a similar situation in another series of antiviral substances. While several simple aliphatic and aromatic glyoxals exhibited antiviral activity in "*in vitro–in vivo*" systems,[5] this activity could not be confirmed *in vivo*.[6] However, this could be overcome in some aromatic glyoxal derivatives by increasing the molecular area; several glyoxals derived from biphenyl,[4b] diphenylmethane, and diphenylethane showed antiviral activity in mice infected with influenza virus A-PR8 strain and hepatitis virus MHV_3 strain. In the absence of further information on the mode of action of such glyoxals, it was deemed worth trying whether a similar molecular increment would produce *in vivo* antiviral activity in DOPA analogs.

For this reason, DL-β-[(2-benzyl-3,4-dihydroxy)-phenyl]-α-alanine and DL-β-[(2-phenyl-3,4-dihydroxy)-phenyl]-α-alanine were synthesized. These compounds were found to be inactive when tested at their maximal toxic concentrations in the following tissue culture systems: Echo 9 and Para-influenza Type III viruses in monkey kidney cells; Vaccinia and Herpes simplex viruses in rabbit kidney cells.[7] In view of these results, they were not tested *in vivo*. The experience in the glyoxal series apparently cannot be transposed to DOPA analogs.

Chemistry.—The starting material for the synthesis of DL-β-[(2-phenyl-3,4-dihydroxy)phenyl]-α-alanine was 3-phenylveratrole,[8] whose preparation was carried out most profitably by dehydrogenating 1-(2,3-dimethoxyphenyl)cyclohexene with chloranil in xylene[9] rather than catalytically.[8] Chloromethylation of 3-phenylveratrole gave 2-phenylveratryl chloride, which was condensed with diethyl acetamidomalonate to give diethyl acetamido-(2-phenylveratryl) malonate. Hydrolysis and ether cleavage with 47% hydrobromic acid led to 2-phenyl-DOPA.

The position of the chloromethyl group in 2-phenylveratryl chloride was proved by oxidation to 2-phenylveratric acid, which was cyclized to 3,4-dimethoxy-9-fluorenone. This reference compound was also synthesized by reaction of 7,8-dimethoxy-2-methyl-4H-3,1-benzoxaz-4-one[10] with phenylmagnesium bromide patterned on the method of Lothrop and Goodwin.[11] An Ullman synthesis[12] using 2-amino-3,4-dimethoxybenzophenone gave 3,4-dimethoxy-9-fluorenone. The melting point of a mixture of the two materials prepared by the two different routes was undepressed, and the infrared spectra of the two samples were superimposable.

For the synthesis of DL-2-benzyl-DOPA, 2,3-dimethoxybenzhydrol[13] was converted to the chloride and this was hydrogenated to 2,3-dimethoxydiphenylmethane. This compound was chloromethylated and converted to β-[(2-benzyl-3,4-dihydroxy)phenyl]-α-alanine by the same types of reactions described for 2-phenyl-DOPA above.

The position of the chloromethyl group (and thereby the ensuing alanine side chain) was ascertained by oxidizing the chloromethyl derivative to 2-benzoylveratric acid, both benzyl carbon atoms being oxidized. Cyclization with sulfuric acid led to 2-methylalizarin (1-hydroxy-2-methoxy-9,10-anthraquinone). The identity of this material was established by comparison with an authentic sample prepared by methylation of alizarin with dimethyl sulfate.[14]

Experimental[15]

2-Phenylveratric Acid.—A solution of 5.35 g. (25 mmoles) of 3-phenylveratrole[9] and 4.03 g. (50 mmoles) of monochloromethyl ether in 17.5 ml. of glacial acetic acid was stirred at 50–55° for 24 hr. and poured into 75 ml. of ice-water. The oily material was extracted into ether, the ether solution was washed with 10% sodium carbonate solution and water, dried, and evaporated. The remaining yellow oily 2-phenylveratryl chloride could not be purified without decomposition either by distillation or chromatography over Darco or alumina.

A stirred solution of 1.5 g. of crude 2-phenylveratryl chloride and 3 g. of potassium permanganate in 50 ml. of water and 1 ml. of a 25% sodium hydroxide solution was refluxed for 1 hr., filtered

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from manganese dioxide, and the filtrate was washed with ether and acidified with dilute hydrochloric acid. The precipitate obtained was filtered and recrystallized from aqueous ethanol. Colorless plates, m.p. 197–198°. Yield, in the oxidation, 0.28 g. (19%).

Anal. Calcd. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46. Found: C, 70.03; H, 5.73.

Diethyl Acetamido-(2-phenylveratryl)-malonate.—To a stirred solution of 0.4 g. (17.5 mmoles) of sodium in 40 ml. of absolute ethanol were added 3.8 g. (17.5 mmoles) of diethyl acetamidomalonate and crude 2-phenylveratryl chloride [prepared from 5.35 g. (2.5 mmoles) of 3-phenylveratrole]. After 9 hr. of refluxing and stirring, the precipitated sodium chloride was filtered and the filtrate evaporated under reduced pressure. The residue was dissolved in ether and the ether solution was washed with water, dried, and evaporated. The oily residue solidified on trituration with ligroin. The crystals were filtered and recrystallized from aqueous ethanol. Colorless needles, m.p. 128.5–129.5°; yield, 2.17 g. (39% based on diethyl acetamidomalonate).

Anal. Calcd. for $C_{24}H_{29}NO_7$: C, 64.99; H, 6.59. Found: C, 65.60; H, 6.60.

DL-β-[(2-Phenyl-3,4-dimethoxy)phenyl]-α-alanine.—A solution of 1 g. (2.26 mmoles) of diethyl acetamido-(2-phenylveratryl)-malonate in 2.5 ml. of glacial acetic acid and 4 ml. of 37% hydrochloric acid was heated at 95° for 6 hr. The mixture was evaporated under reduced pressure and the residue treated with dry acetone. The resulting crystals were filtered and recrystallized from a mixture of absolute ethanol and ethyl acetate. The colorless leaflets of DL-β-[(2-phenyl-3,4-dimethoxy)phenyl]-α-alanine hydrochloride (0.25 g., 33%) melted at 227–228° dec. (evacuated capillary).

Anal. Calcd. for $C_{17}H_{19}NO_4 \cdot HCl$: C, 60.44; H, 5.96. Found: C, 60.03; H, 5.75.

DL-β-[(2-Phenyl-3,4-dihydroxy)phenyl]-α-alanine.—A solution of 10 g. (0.023 mole) of diethyl acetamido-(2-phenylveratryl) malonate in 47 ml. of redistilled 47% hydrobromic acid containing a trace of sodium hypophosphite was refluxed for 7 hr. while nitrogen was bubbled through. The solution was evaporated under reduced pressure at 90°. The residue was dissolved in a small amount of water containing a trace of sodium bisulfite and the amino acid was liberated by adjusting to pH 5–6 with a saturated solution of potassium carbonate. After standing for several days, the material crystallized. It was recrystallized from water containing a trace of sulfur dioxide. Colorless matted needles, m.p. 178–182° dec.; yield, 3.5 g. (57%).

Anal. Calcd. for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.12. Found: C, 65.92; H, 5.83; N, 5.19.

2-Amino-3,4-dimethoxybenzophenone.—A solution of phenylmagnesium bromide [from 12.4 g. (7.9 mmoles) of bromobenzene] in 200 ml. of anhydrous ether was added, under a nitrogen atmosphere, over a 30-min. period, to a stirred ice-cold suspension of 17.46 g. (7.9 mmoles) of 7,8-dimethoxy-2-methyl-4H-3,1-benzoxaz-4-one[10] in 200 ml. of anhydrous benzene. The mixture was stirred for another 7 hr. at 26° and then hydrolyzed with dilute hydrochloric acid. After removal of the solvent by steam distillation, the solid residue was filtered, washed with water, and refluxed for 3 hr. in 100 ml. of ethanol and 20 ml. of concentrated hydrochloric acid. The mixture was treated with charcoal, made ammoniacal, and diluted with 300 ml. of water. The resulting solid was taken up in ether, washed with water, and the ether was removed. The solid residue was recrystallized from dilute ethanol. The pale yellow leaflets of m.p. 112–112.5° weighed 6.8 g. (34%).

Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.87. Found: C, 69.73; H, 5.70.

The **2,4-dinitrophenylhydrazone,** red needles from ethanol–ethyl acetate, melted at 222.5–224° dec. (uncor.).

Anal. Calcd. for $C_{21}H_{19}N_5O_6$: C, 57.66; H, 4.37; N, 16.01. Found: C, 57.55; H, 4.21; N, 16.75.

3,4-Dimethoxy-9-fluorenone. A.—From 2-Phenylveratric Acid.—A mixture of 1.53 g. (5.9 mmoles) of 2-phenylveratric acid and 5 ml. of thionyl chloride was refluxed for 30 min., excess thionyl chloride was removed under reduced pressure, and the residual oil dissolved in 75 ml. of ether. After washing with 10% sodium hydroxide solution and water, the ether solution was dried and evaporated. Recrystallization of the residue from methanol gave 0.42 g. (30%) of yellow needles, m.p. 145.5°.

Anal. Calcd. for $C_{15}H_{12}O_3$: C, 74.98; H, 5.03. Found: C, 75.04; H, 5.16.

B. From 2-Amino-3,4-dimethoxybenzophenone.—A solution of 1 g. (3.9 mmoles) of 2-amino-3,4-dimethoxybenzophenone in 3.5 ml. of 98% sulfuric acid and 6.4 ml. of water was cooled to −5° to 0° and diazotized with a solution of 0.27 g. (3.9 mmoles) of sodium nitrite in a few drops of water. After heating on a steam bath for 2.5 hr., the mixture was cooled, diluted with 30 ml. of water, and extracted with ether. The ether extract was washed, dried, and worked up. The solid residue was chromatographed on aluminum oxide, eluted with benzene, and recrystallized from methanol. A small yield of yellow needles was obtained, m.p. 145.5–146°, undepressed by admixture with a sample obtained by method A.

The **2,4-dinitrophenylhydrazone** consisted of red needles (from nitrobenzene), m.p. 290–292° dec.

Anal. Calcd. for $C_{21}H_{16}N_4O_8$: C, 59.99; H, 3.83; N, 13.32. Found: C, 60.13; H, 3.86; N, 13.64.

2,3-Dimethoxydiphenylmethane.—A solution of 112 g. of 2,3-dimethoxybenzhydrol[13] in 250 ml. of concentrated hydrochloric acid was refluxed for 8 hr., cooled, poured into ice–water, and extracted three times with chloroform. The extracts were washed with an ice-cold 5% solution of sodium bicarbonate, dried over magnesium sulfate, and evaporated under vacuum. The yellow oily residue was treated with charcoal in dry benzene in the cold overnight and recovered by filtration and vacuum evaporation of the solvent. The infrared spectrum showed a band at 680 cm.$^{-1}$ (C–Cl) but no O–H stretching band. The oil was hydrogenated (10% Pd/C) in ethyl acetate solution at 3.09 kg./cm.^{2} pressure (1 hr.). The filtered solution was fractionated; the colorless distillate (74.4 g., 66%) had b.p. 121° (0.25 mm.), $\alpha^{24}D$ 1.5693.

Anal. Calcd. for $C_{15}H_{16}O_2$: C, 78.92; H, 7.07. Found: C, 78.35; H, 7.02.

2-Benzylveratric Acid.—A mixture of 20 g. of 2,3-dimethoxydiphenylmethane, 14 g. of monochloromethyl ether, and 70 ml. of glacial acetic acid was stirred at 50–55° for 24 hr., poured into 300 ml. of ice–water, and extracted with chloroform. The extracts were washed with ice-cold 10% sodium carbonate solution, dried over magnesium sulfate, and evaporated. The oily 2-benzylveratryl chloride, b.p. 154° (0.3 mm.), weighed 17.5 g. (72%) but was too unstable to be purified further.

By refluxing 2 g. of 2-benzylveratryl chloride with 50 ml. of 10% aqueous sodium hydroxide solution for 1 hr., a solid 2-benzylveratryl alcohol was obtained. It was filtered from the cooled mixture and oxidized in 100 ml. of refluxing chloroform with 10 g. of manganese dioxide for 24 hr. The manganese dioxide was filtered off and the chloroform evaporated. The infrared spectrum of the residual oil showed an aldehyde peak, and therefore the oil was oxidized further with a 3% potassium permanganate solution. The mixture was filtered, and the carboxylic acid was obtained on acidification of the filtrate. It was recrystallized from aqueous ethanol; colorless crystals (0.5 g.), m.p. 146–148°.

Anal. Calcd. for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92. Found: C, 70.42; H, 5.99.

2-Benzoylveratric acid.—By prolonging the permanganate oxidation just described, the keto acid, m.p. 188–190° (from aqueous ethanol) was obtained as fine colorless needles.

Anal. Calcd. for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found: C, 67.56; H, 5.07.

2-Methylalizarin.—A mixture of 200 mg. of 2-benzoylveratric acid and 5 ml. of concentrated sulfuric acid containing 8% of sulfur trioxide and 10% of boric acid was heated at 90° for 10 min. The solution turned dark red immediately. It was poured into ice, and the precipitated yellowish material was filtered and recrystallized from ethanol. The orange needles (40 mg.) melted at 230–231°; this m.p. was not depressed by admixture with an authentic sample prepared from alizarin.[14]

Anal. Calcd. for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96. Found: C, 70.69; H, 3.94.

Diethyl Acetamido-(2-benzylveratryl)-malonate.—2-Benzylveratryl chloride (5.54 g., 0.02 mole) was added to a stirred solution of 0.46 g. (0.02 mole) of sodium in 45 ml. of absolute ethanol. Then 4.34 g. (0.02 mole) of diethyl acetamidomalonate was added and the mixture stirred and refluxed for 24 hr. The solution was filtered from sodium chloride and the filtrate evaporated under reduced pressure. The oily residue solidified on treatment with petroleum ether. It was recrystallized from aqueous ethanol; the colorless crystals (5.58 g., 61%) had m.p. 111–112.5°.

Anal. Calcd. for $C_{25}H_{31}NO_7$: C, 65.63; H, 6.83. Found: C, 65.46; H, 6.59.

DL-β-[(2-Benzyl-3,4-dihydroxy)phenyl)]-α-alanine.—A mix-

ture of 1.5 g. of ethyl acetamido-2-(benzylveratryl)-malonate and 10 ml. of 47% hydriodic acid, stabilized with hypophosphite, was refluxed under a slow stream of nitrogen for 9 hr. Excess hydriodic acid was distilled off *in vacuo* at below 95°. The yellow viscous oily residue was dissolved in a minimum of water, and the amino acid was liberated by adjusting to pH 6 with a saturated potassium carbonate solution. The colorless solid was recrystallized from dimethylformamide, m.p. 246–248° dec. The yield was 0.70 g. (71.5%).

Anal. Calcd. for $C_{19}H_{17}NO_4$: C, 66.88; H, 5.96. Found: C, 66.79; H, 6.22.

Extensive decomposition occurred when freshly distilled and hypophosphite-stabilized hydrobromic acid was substituted for hydriodic acid in this experiment.

Communications to the Editor

The Tumor-Enhancing and Irritant Principles from *Croton tiglium L.*

Sir:

Croton oil is obtained from *Croton tiglium L.* (Euphorbiaceae) by pressing of the seed and has long been known for its vesicant, toxic, and purgative activity.[1] In 1941 Berenblum[2] showed that this material is also a potent tumor-enhancing agent, *i.e.*, it stimulates the appearance and rapid growth of tumors on mouse epidermis pretreated with a minute dose of a carcinogenic hydrocarbon. At the low concentration used the hydrocarbon, when applied alone, is inactive and croton oil itself is not carcinogenic. This unique activity of croton oil has important implications in studies of cancer causation and many attempts have been made to isolate and determine the structure of the active components.[1,3-5] Tumor-enhancing agents of known chemical constitution are all much less active than croton oil.[6]

The present report deals with the isolation of two active materials and the structure elucidation of a pure crystalline material obtained by catalytic hydrogenation of a biologically active fraction of croton seed extract.

The shelled seed of *Croton tiglium L.* was extracted with methyl alcohol to give a 41% yield of an oily extract. The vesicant fraction of the extract, croton resin (I), was prepared by solvent partition between aqueous methyl alcohol (1:9) and hexane.[1] This polar mixture of compounds (3% of whole extract) has shown potent tumor-enhancing activity[2] and it was therefore chosen for further chemical studies and biological assay. Thin layer chromatography of I on silica gel showed the presence of at least 14 components. Column chromatography of I on acid-washed florisil gave an amorphous solid (II, 1.0% of whole extract). Both I and II showed a pronounced increase in tumor-enhancing activity compared to that of the whole croton seed extract or commercial croton oil.[7] Thin layer chromatography of II showed the presence of four components (A, B, C, and D). This material was further fractionated by countercurrent distribution and thin layer chromatography to give two biologically active amorphous materials, A and C, which showed single spots on thin layer chromatograms. Both A and C are irritant and show tumor-enhancing activity. Compound C was tested on 20 female Swiss Millerton mice initiated with 300 γ of 7,12-dimethylbenz[*a*]anthracene. The promotor (C) was applied three times weekly, 5 γ per application. First tumors were observed at 44 days after initiation and at 68 days 17 animals bore tumors. Tumors were not observed in appropriate control groups.

All the components of II are unsaturated and can be hydrogenated with palladium black as catalyst in methyl alcohol solution. Catalytic hydrogenation of II followed by countercurrent distribution (lower phase: aqueous methyl alcohol, 0.5:9.5, containing 0.4% glacial acetic acid; upper phase: hexane; 200 tubes, 10 ml. per phase, 400 transfers) gave a 50% yield of hydrogenation product which was purified by chromatography on silica gel plates (2 mm. thickness.)[8] The band with the highest R_f was eluted with ether and gave a crystalline product from ethyl alcohol–water; colorless needles, m.p. 96°, R_f 0.25 (hexane–ether–acetic acid, 9:1.5:0.5, silica gel). The analytical data agree with a molecular formula, $C_{36}H_{60}O_8$, molecular weight 620, with three ester functions, two free hydroxyl groups, and six carbon-methyl groups. The compound is optically active, $[\alpha]D^{21}$ +124.6° (*l* = 1 dm., *c* 0.77, chloroform).

The ultraviolet absorption spectrum showed two low-intensity maxima in cyclohexane and in methyl alcohol (cyclohexane: λ_{max} 248 mμ; ϵ_{max} 410; λ_{max} 302 mμ; ϵ_{max} 56) which suggested the presence of a polysubstituted benzene ring.

The infrared absorption spectrum was examined in various media and revealed the following features: intramolecularly bonded hydroxyl (0.001 *M* in carbon tetrachloride: a weak band at 3570 cm.$^{-1}$ and a strong sharp band at 3440 cm.$^{-1}$); intense aliphatic stretching and bending absorptions (KBr pellet, methyl and methylene at 2965, 2935, 2860, 1465, and 1370 cm.$^{-1}$; tertiary –CH at 2885 cm.$^{-1}$); intense ester carbonyl absorption (KBr pellet, 1742, 1720 cm.$^{-1}$; 0.001 *M* in carbon tetrachloride, 1735 cm.$^{-1}$); weak aromatic C=C absorption (0.05 *M* in chloroform, 1600 cm.$^{-1}$). Aliphatic C=C absorption is present in II, A, and C at 1650 and 1630 cm.$^{-1}$ but does not appear in the infrared spectrum of the crystalline hydrogenated compound. However, the other bands in the infrared spectra of A and C are very similar to that of the hydrogenated compound.

The n.m.r. spectrum showed a series of four peaks from 0.9 to 1.25 p.p.m. ascribed to aliphatic CH, CH_2,

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