(45%) of colorless 3-(*o*-phenetidino)propionitrile, b.p. 141–143°/0.7 mm., n_D^{25} 1.5476. The analytical values are reported in Table II.

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[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH COLLEGE OF MEDICINE]

Convenient Synthesis for β-(3-Indolyl)-DL-Lactic Acids[1]

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A convenient synthesis of β-(3-indolyl)-, β-[3-(5-methoxyindolyl)]-, and β-[3-(5-benzyloxyindolyl)]-DL-lactic acids is described.

Many of the procedures used to synthesize α-hydroxy acids cannot be used with compounds containing an indole group because of the chemical reactivity of this nucleus toward acidic and oxidizing conditions. Thus, the usual methods which involve halogenation of β-substituted-α-carboxypropionic acids followed by decarboxylation and hydrolysis of the α-halo acids in the manner used for the preparation of β-phenyllactic acid,[2] or known conversions of α-amino acids to the α-hydroxy acids *via* the diazonium salt by treatment with nitrous acid,[3] nitrosyl bromide,[4] or silver nitrite[5] do not seem applicable as convenient preparative routes to indolelactic acids. Syntheses from the appropriately substituted aldehydes *via* the cyanohydrin as an intermediate[6] are precluded because of the difficulty of preparation and the instability of some indole-substituted acetaldehydes.[7]

β-(3-Indolyl)lactic acid, itself, appears to be the only indolelactic acid which has been prepared. This compound was first made by the biological conversion of L-tryptophan to β-(3-indolyl)-D-lactic acid by the mold *Oidium lactis*;[8] the racemic compound has been prepared from this product by racemization with alkali.[9,10] The completely synthetic approaches to indolelactic acid all have involved the use of indolepyruvic acid as an intermediate; it is readily converted to indolelactic acid by reduction with sodium amalgam.[9] The chemical instability of indolepyruvic acid itself[11,12] indicated that the use of substituted indolepyruvic acids as intermediates would be impractical unless they could be prepared readily and in good yield from available precursors. Consequently, a more direct route to indolelactic acid and substituted indolelactic acids was sought.

Tryptophan and some of its derivatives have been prepared conveniently by the condensation of gramine and substituted gramines with various aminomalonate derivatives using alkaline catalysis.[13,14] By analogy with this reaction, the condensation of gramine (I) with diethyl acetoxymalonate (II) was investigated and was found to proceed smoothly. Hydrolysis of the product (III) yielded β-(3-indolyl)-α-carboxy-α-hydroxypropionic acid (IV), which was decarboxylated to give indolelactic acid (V), in a yield of 52%, based on I.

With a similar sequence of reactions, 5-methoxyindolelactic acid (VI) was prepared from 5-methoxygramine (VII), and 5-benzyloxyindolelactic acid (VIII) from 5-benzyloxygramine (IX). 5-Hydroxyindolelactic acid (X), which is of interest as a possible metabolite of 5-hydroxytryptophan, was prepared by catalytic hydrogenation of VIII. It was not possible to obtain X crystalline, but paper chromatography showed the compound to be homogeneous and to have the expected properties.

The generality of this procedure for the preparation of other substituted lactic acids was

(1) Supported by research grants from the National Institutes of Health, U. S. Public Health Service.

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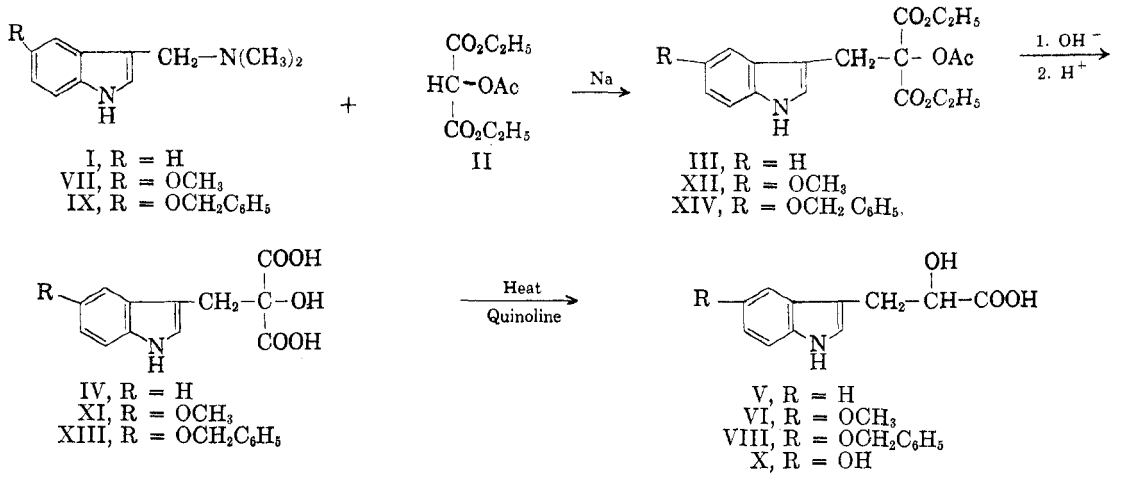

I, R = H
VII, R = OCH_3
IX, R = $OCH_2C_6H_5$

II

III, R = H
XII, R = OCH_3
XIV, R = $OCH_2C_6H_5$

IV, R = H
XI, R = OCH_3
XIII, R = $OCH_2C_6H_5$

V, R = H
VI, R = OCH_3
VIII, R = $OCH_2C_6H_5$
X, R = OH

tested in an attempted synthesis of β-phenyllactic acid. The condensation of II with *N,N*-dimethylbenzylamine[15] (sodium as the condensing agent) followed by saponification of the crude product and decarboxylation (in quinoline) of the crude dicarboxylic acid afforded only a very small amount of phenyllactic acid. By using benzyl chloride[16] in place of the amine (sodium ethoxide as the condensing agent) some improvement in the yield of phenyllactic acid was observed, but it appears that the method, as it presently exists, is not satisfactory for the preparation of phenyllactic acid. An extensive study of the preparation of other lactic acids with this procedure was not conducted since most of them can be prepared more readily by other methods.

EXPERIMENTAL[17]

Diethyl acetoxymalonate (II).[18] A solution of 80 g. (0.5 mole) of diethyl malonate in 200 ml. of glacial acetic acid was placed in a 1-l. three-necked flask fitted with a stirrer, condenser, and an Erlenmeyer flask attached with a rubber sleeve, and was heated to 100° in an oil bath. To the hot solution was added, with stirring, 217.3 g. (0.49 mole) of lead tetraacetate in small portions (by means of the attached Erlenmeyer flask) at such a rate that a gentle reflux was maintained (20 min.). After the addition was completed, the mixture was maintained at 100–105° for 1.5 hr. The acetic acid was then removed by distillation *in vacuo*. To the resulting white pasty mass was added 300 ml. of water, and the mixture was extracted with ether (4 × 100 ml.). The combined ether extracts were washed successively with saturated sodium bicarbonate solution (4 × 100 ml.) and a 25% solution of sodium sulfate and dried over anhydrous sodium sulfate. The ether was removed and the residual yellow oil was distilled *in vacuo*. The yield of pure II was 69.2 g. (65% based on lead tetraacetate), b.p. 137–138°/17 mm., n_D^{30} 1.4200.

β-(3-Indolyl)-α-hydroxy-α-carboxypropionic acid (IV). A mixture of 17.42 g. (0.10 mole) of I, 32.73 g. (0.15 mole) of II, 0.07 g. (0.003 g.-atom) of sodium, and 150 ml. of toluene was heated at reflux temperature while a slow stream of nitrogen was bubbled through the solution. Completion of reaction, as indicated by the cessation of the evolution of dimethylamine (moist *p*H paper), required about 20 hr. The mixture was cooled, the unreacted sodium was removed, and the solution was poured into 300 ml. of water containing 15 ml. of concentrated hydrochloric acid. The mixture was extracted with ether (4 × 100 ml.), and the combined ether extracts were washed successively with a saturated solution of sodium bicarbonate and a 25% solution of sodium sulfate. The ether solution was dried over anhydrous sodium sulfate, and the solvent was removed by distillation; the residual amber-colored oil (47.2 g.) consisted of a mixture of II and III. Because extensive attempts to crystallize II were unsuccessful, the crude mixture was saponified in the following manner to yield IV. To the crude mixture of II and III was added a solution of 40 g. (1.0 mole) of sodium hydroxide in 200 ml. of water and 50 ml. of ethanol, and the mixture was heated at reflux temperature for 6.5 hr. The condenser was then set for distillation and 75 ml. of distillate was collected and discarded. The residual alkaline solution was cooled, extracted with ether (2 × 200 ml.) and acidified to *p*H 1 by the dropwise addition of cold dilute hydrochloric acid to the rapidly stirred ice-cold solution. The turbid solution was extracted with ethyl acetate (4 × 100 ml.), the combined extracts were dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* (bath temp. below 40°). The residual brown oil (28.4 g.) was mixed with 100 ml. of ethylene dichloride and crystallization was induced by scratching. The resulting slurry was cooled to room temperature and finally to −5° for several hours. The product was collected, washed with 50 ml. of cold ethylene dichloride and dried. The pink, rectangular prisms of IV amounted to 17.4 g. (70% based on I), m.p. 163–165° (dec.). The combined filtrate and ethylene dichloride washings from the first crop were concentrated to dryness *in vacuo*, but only 0.7 g. more of crude IV was obtained.

The 17.4 g. of IV was recrystallized from a mixture of 60 ml. of ethylene dichloride and 47 ml. of glacial acetic acid to yield pure IV; 11.3 g. (45% based on I), m.p. 163° (dec.). The mother liquor was reworked to yield an additional 2.0 g., m.p. 158° (dec.).

A sample recrystallized for analysis melted at 165° (dec.).

Anal. Calcd. for $C_{12}H_{11}NO_5$: C, 57.82; H, 4.45; N, 5.62. Found: C, 57.08; H, 4.24; N, 5.45.

β-(3-Indolyl)-DL-lactic acid (V). A mixture of 2.49 g. (0.01 mole) of IV, 12.9 g. (0.10 mole) of quinoline (redistilled)[19] and 0.1 g. of copper powder was heated at 125° for 45 min. under a slow stream of nitrogen; the temperature was then raised to 145° and maintained for an additional hour. The resulting straw-colored solution was cooled,

(15) Compare with H. R. Snyder, E. L. Eliel, and R. E. Carnahan, *J. Am. Chem. Soc.*, **72**, 2958 (1950).

(16) Similar procedure used for preparation of diethyl benzylmalonate; *Org. Syntheses*, **Coll. Vol. III**, 705 (1955).

(17) All melting and boiling points are uncorrected.

(18) O. Dimroth and R. Schweizer, *Ber.*, **56**, 1380 (1923).

(19) Much lower yields are obtained if unpurified quinoline is used.

poured into 60 ml. of cold 2*N* hydrochloric acid, and the resulting solution was saturated with sodium sulfate and extracted with ethyl acetate (5 × 100 ml.). The combined ethyl acetate extracts were extracted with 5% sodium bicarbonate solution (6 × 25 ml.). The combined bicarbonate extracts were cooled in ice and acidified to *p*H 1.5 by the dropwise addition of cold 4*N* hydrochloric acid. The slurry that resulted was saturated with sodium sulfate and extracted with ethyl acetate (4 × 100 ml.). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* to yield 1.82 g. (89%) of V, m.p. 140–144°. The crude product was recrystallized from 175 ml. of ethylene dichloride (Norite) to yield 1.54 g. (75% based on IV) of white, glistening plates, m.p. 146–147°, undepressed on admixture with authentic DL-indolelactic acid.[9]

β-[3-(5-Methoxyindolyl)]-α-hydroxy-α-carboxypropionic acid (XI). A mixture of 1.27 g. (0.006 mole) of VII[20] (m.p. 124–125°), 2.18 g. (0.01 mole) of II, a 1 mm.[3] piece of sodium and 15 ml. of toluene was heated at reflux temperature while a slow stream of nitrogen was bubbled through the solution until the evolution of dimethylamine had ceased (about 20 hr.). The mixture was cooled, unreacted sodium was removed, and the straw-colored solution was poured into 50 ml. of cold 0.25*N* hydrochloric acid. Treatment of the mixture in a manner similar to that described for the preparation of IV afforded 3.31 g. of crude XII. The crude ester was suspended in a solution of 3.0 g. (0.075 mole) of sodium hydroxide in 50 ml. of water and 15 ml. of ethanol and the mixture was heated under reflux for 6 hr. Treatment of the reaction mixture in a manner similar to that described for the preparation of IV afforded 2.17 g. of a yellow oil which crystallized upon manipulation after the addition of a small amount of ethylene dichloride. The crude product amounted to 1.83 g. (106% based on VII). Recrystallization from a mixture of 15 ml. of ethylene dichloride and 3 ml. of glacial acetic acid yielded 1.12 g. (65% based on VII) of XI, pink rectangular prisms, m.p. 152° (dec.).

A sample recrystallized for analysis melted at 154° (dec.).

Anal. Calcd. for $C_{13}H_{13}NO_6$: C, 55.91; H, 4.69; N, 5.02. Found: C, 55.85; H, 4.59; N, 5.24.

β-[3-(5-Methoxyindolyl]-DL-lactic acid (VI). A mixture of 1.23 g. (0.0044 mole) of XI, 12.9 g. (0.1 mole) of redistilled quinoline and 0.1 g. of copper powder was heated in an oil bath at 155° for 1 hr. while a stream of nitrogen was passed over the solution. The temperature was then gradually raised to 170° over a period of 30 min. (evolution of CO_2 ceased after the first hour of heating at 155°). Treatment of the resulting straw-colored reaction mixture in a manner similar to that described for the preparation of V afforded a yellow oil which crystallized after the addition of a few drops of ethylene dichloride followed by scratching. The crude, tan-colored solid was collected and dried; yield, 0.74 g. (72%), m.p. 126–128°. Recrystallization from a mixture of 8 ml. of ethylene dichloride and 1 ml. of glacial acetic acid afforded 0.59 g. of light buff-colored rectangular prisms of VI, m.p. 128–129°.

Anal. Calcd. for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.96. Found: C, 61.70; H, 5.72; N, 5.80.

β-[3-(5-Benzyloxyindolyl)]-α-hydroxy-α-carboxypropionic acid (XIII). With a procedure similar to that used for the preparation of IV, 7.01 g. (0.025 mole) of IX,[14] 8.73 g. (0.040 mole) of II, and three small pieces (1 mm.[3]) of sodium in 75 ml. of toluene were allowed to react, and the mixture was worked up to yield 18.74 g. of crude XIV. The crude XIV was suspended in a solution of 12.0 g. (0.30 mole) of sodium hydroxide in 100 ml. of water and 50 ml. of ethanol, and the mixture was heated at reflux temperature for 6.5 hr. The condenser was set for distillation; 75 ml. of distillate was collected and discarded, and the residual alkaline solution was extracted with ether (2 × 200 ml.). The aqueous phase was then cooled in ice and acidified to *p*H 1 by the careful addition of cold dilute hydrochloric acid. The mixture was then extracted with ethyl acetate (4 × 100 ml.), and the combined ethyl acetate extracts were extracted with saturated sodium bicarbonate solution (4 × 100 ml.). The combined bicarbonate extracts were treated with Norite (room temperature) and filtered through Celite. The amber filtrate was cooled in ice and acidified carefully (CO_2 evolved) to *p*H 1.8 by the careful addition of 6*N* hydrochloric acid. The resulting mixture was extracted with ethyl acetate (4 × 100 ml.), the combined extracts were dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* (bath temp. below 45°). The red-brown oil which was obtained crystallized to a paste when seeded with some crystals obtained by scratching a small portion of the oil suspended in ethylene dichloride. The paste was washed three times with 5-ml. portions of cold nitroethane. The crude product amounted to 5.81 g. (66% based on IX), m.p. 140° (dec.). After recrystallization from 15 ml. of nitroethane, 4.50 g. (51%) of gray XIII was obtained, m.p. 140° (dec.).

A sample was recrystallized for analysis from a mixture of ethylene dichloride and glacial acetic acid (25:1), m.p. 140° (dec.).

Anal. Calcd. for $C_{19}H_{17}NO_6$: C, 64.22; H, 4.82; N, 3.94. Found: C, 63.72; H, 4.75; N, 3.80.

β-[3-(5-Benzyloxyindolyl)]-DL-lactic acid (VIII). A mixture of 2.0 g. (0.0056 mole) of XIII, 27 g. (0.23 mole) of redistilled quinoline, and 0.1 g. of copper powder was heated at 200° for 45 min. under a slow stream of nitrogen. The clear, straw-colored solution was cooled and poured into 125 ml. of cold 2.5*N* hydrochloric acid and treated in a manner similar to that described for the preparation of V. The resulting oil crystallized to a light tan solid; 1.60 g. (92%). Recrystallization from 10 ml. of ethylene dichloride afforded 1.44 g. of small, tan, rectangular prisms of VIII, m.p. 121–122°.

A sample recrystallized for analysis melted at 124–125°.

Anal. Calcd. for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 70.17; H, 5.39; N, 4.41.

β-[3-(5-Hydroxyindolyl)]-DL-lactic acid (X). A mixture of 0.31 g. (0.001 mole) of VIII, 0.02 g. of 5% Pd/C catalyst, and 15 ml. of 95% ethanol was subjected to hydrogenation at atmospheric pressure and room temperature for 6.5 hr. The catalyst was removed by filtration under nitrogen and the ethanol solution was concentrated to a small volume (2 to 3 ml.). Attempts to isolate a solid product resulted in rapid decomposition with the formation of colored oils. Paper chromatographic studies on the ethanol concentrate indicated that pure X was obtained; R_F 0.28, isopropyl alcohol-aq. NH_3-H_2O, 8:1:1; maroon color with diazotized sulfanilic acid,[21] blue color with acidic *p*-dimethylaminobenzaldehyde,[21] dark gray with ammoniacal silver nitrate,[21] and an immediate lavender color with a α-nitrosonaphthol reagent.[22]

DL-β-Phenyllactic acid: Procedure A. (Condensation of II with *N,N*-dimethylbenzylamine.) A mixture of 6.76 g. (0.05 mole) of *N,N*-dimethylbenzylamine, 16.36 g. (0.075 mole) of II, a trace (piece 1 mm.[3]) of sodium, and 40 ml. of toluene was heated at reflux temperature under a stream of nitrogen for 24 hr. Evolution of volatile amine was not detectable. The toluene was removed and the residue then heated at 185–190° for an additional 36 hr. (The evolution of volatile amine was observed with moist *p*H paper.) Treat-

(20) Prepared in 75% yield by a method similar to that described for the preparation of 5-benzyloxygramine; See ref. 14. Compare with J. B. Bell, Jr., and H. G. Lindwall, *J. Org. Chem.*, **13**, 547 (1948).

(21) H. K. Berry, H. E. Sutton, L. Cain, and J. S. Berry, *Univ. Texas Pub.*, No. 5109, 22 (1951).

(22) S. Udenfriend, H. Weissbach, and C. T. Clark, *J. Biol. Chem.*, **215**, 337 (1955).

ment of the dark brown reaction mixture in a manner similar to that described for IV afforded 24.7 g. of a dark red-brown oil. Saponification of this oil with alcoholic sodium hydroxide followed by acidification and decarboxylation afforded 3.7 g. of a yellow oil. On treatment with benzene there was obtained 0.18 g. (2% based on amine) of DL-β-phenyllactic acid, m.p. 91–94°, undepressed on admixture with authentic DL-β-phenyllactic acid.

Procedure B. (Condensation of II with benzyl chloride.) From a mixture of 22.70 g. (0.104 mole) of II, 12.64 g. (0.100 mole) of benzyl chloride, 2.30 g. (0.100 g.-atom) of sodium, and 50 ml. of absolute ethanol heated at reflux temperature for 67 hr. was obtained 24.8 g. of a yellow oil. Saponification of this oil with alcoholic sodium hydroxide followed by acidification and decarboxylation of the dicarboxylic hydroxy acid with quinoline (at 105°) afforded 1.65 g. (10% based on benzyl chloride) of DL-β-phenyllactic acid, m.p. 93–95°.

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[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

3,16β-Dihydroxy-$\Delta^{1,3,5}$-estratrien-17-one and Related Compounds[1]

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The preparation of 3,16β-dihydroxy-$\Delta^{1,3,5}$-estratriene-17-one (IIb) and the diacetate (IIa) are described. Reduction with lithium aluminum hydride yielded $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol (IVb). The factors involved in the reduction of ring D ketols are briefly discussed.

In view of the fact that ring D ketols appear to be potential intermediates in the biochemical transformation of the estrogenic hormone as well as end products of its metabolism, satisfactory syntheses for these compounds were desired. In a prior report from these laboratories a ready synthesis for 3,16α-diacetoxy-$\Delta^{1,3,5}$-estratriene-17-one (IIIa) was described and this compound was employed as an intermediate in the preparation of estriol.[2] In the present communication, synthesis of the epimeric 3,16β-dihydroxy-$\Delta^{1,3,5}$-estratriene-17-one (IIb) and a means for preparation of the naturally occurring metabolite, $\Delta^{1,3,5}$-estratriene-3,16β,17β-triol (IVb) are described. At the same time it was possible to characterize IIIb more completely and, as an extension of the investigation, to identify a previously reported minor reaction product obtained from the lithium aluminum hydride reduction of IIIa.

The preparation of IIa is a direct application of the investigations of Johnson, Gastambide and Pappo[3] who described a stereoselective oxidation of the enol acetate of isoandrosterone to yield 3β,16β-diacetoxyandrostane-17-one. When the reaction conditions described by these authors were employed with estrone-enol diacetate (I) a 42% yield of IIa was obtained. The compound crystallized in at least two polymorphic modifications but the physical constants, and especially the infrared spectrum clearly distinguished the product from IIIa. The compound was readily rearranged by means of either alkali or acid to the stable isomer 3,17β- dihydroxy- $\Delta^{1,3,5}$ -estratriene- 16 -one (VIb). Reduction of IIa by means of lithium aluminum hydride yielded only the known $\Delta^{1,3,5}$-estriene-3,16β,17β-triol (IVb).[4] Despite intensive search no evidence was obtained for the presence of the as yet undescribed fourth isomer of estriol, *i.e.* $\Delta^{1,3,5}$-estratriene-3,16β,17α-triol.

The virtual stereospecificity observed in the metal hydride reduction of IIa may be ascribed to the interposition of the C-18 methyl group and the complex of the reagent with the C-16β-oriented oxygen to the approach of the hydride toward C-17. On the other hand, when IIIa was reduced with lithium aluminum hydride, in addition to the major product, estriol, about 10% of the epimeric $\Delta^{1,3,5}$-estratriene-3,16α,17α-triol (Vb) was formed. The latter was reported earlier[2] as an unidentified component in the reduction of IIIa. The lesser stereoselectivity toward lithium aluminum hydride in the case of IIIa is explicable in the same terms except that the C-16 hydroxyl is on the opposite side of the molecule. The transposition of this group may facilitate formation of the 17α-hydroxy isomer both by removal of a shield over the β face of C-17 and through a cyclic complex of the metal with the two oxygen atoms at C-16 and C-17. The C-18 methyl group appears to be an important factor in obstructing the β attack of C-17 by the metal hydride since reduction of estrone by

(1) This investigation was supported in part by a grant from the American Cancer Society, and a research grant (CY-3207) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

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