3-(Tetrahydropyran-2-yloxy)-oxindole-3-propionic Acid (X) from VIII.—Nine and thirty-two hundredths grams (0.04 mole) of the tetrahydropyranyl ether of dioxindole (VIII) was added to a solution of 1 g. of sodium in 30 ml. of absolute ethanol. This mixture was warmed gently in a nitrogen atmosphere to effect solution. To this solution was carefully added 5 ml. of ethyl acrylate (exothermic reaction). The reaction mixture was then refluxed for 2 hours. The solution was refluxed an additional hour under nitrogen after the addition of 2.6 g. of sodium hydroxide in 20 ml. of water. The solution was then concentrated to remove the alcohol, acidified with concentrated hydrochloric acid and extracted with two 50-ml. portions of methylene chloride. The methylene chloride solution was concentrated to a mobile oil (12.85 g.). Attempts to isolate the 3-(tetrahydropyran-2-yloxy)-oxindole-3-propionic acid (X) were unsuccessful.

Dioxindole-3-propionic Acid (XII) from X.—The yellow oil X was dissolved in 50 ml. of anhydrous ether and 10 drops of 18% hydrochloric acid was added. This solution precipitated an oil which crystallized on chilling and swirling. The product was filtered and washed with ether. Five and two-hundredths grams of dioxindole-3-propionic acid (XII) was obtained, m.p. 176–180°, with some effervescence, which gave no depression when mixed with the Kendall preparation. A 57% yield was obtained from 3-(tetrahydropyran-2-yloxy)-oxindole (VIII). The analytical sample was recrystallized from water and a fine powder, m.p. 195–196°, was obtained.

Anal. Calcd. for $C_{11}H_{11}O_4N$: C, 59.72; H, 5.01. Found: C, 59.98; H, 4.78.

Kendall's Preparation of Dioxindole-3-propionic Acid (XII) from Oxindole-3-propionic Acid (XI).—Two and five-hundredths grams (0.01 mole) of oxindole-3-propionic acid (XI) was dissolved in 10 ml. of 1.0 *N* sodium hydroxide and cooled to 10°. Two equivalents (2.54 g.) of finely divided iodine was added; 6 ml. of 5 *N* sodium hydroxide was then added slowly with swirling. The solution was allowed to come to room temperature and 8 ml. of 5 *N* sulfuric acid was added followed by 0.71 g. of iodic acid in 10 ml. of water. The mixture was then warmed gently to coagulate the iodine, filtered, and the filtrate was concentrated *in vacuo* until crystallization began. The crystals which separated on cooling were filtered and washed with water. Two grams of dioxindole-3-propionic acid (XII), m.p. 178–180°, (yield 90%) was obtained. The product when recrystallized from water gave a fine crystalline powder, m.p. 195–196°.

FRANKLIN PARK, ILLINOIS

[CONTRIBUTION FROM THE JULIAN LABORATORIES, INC.]

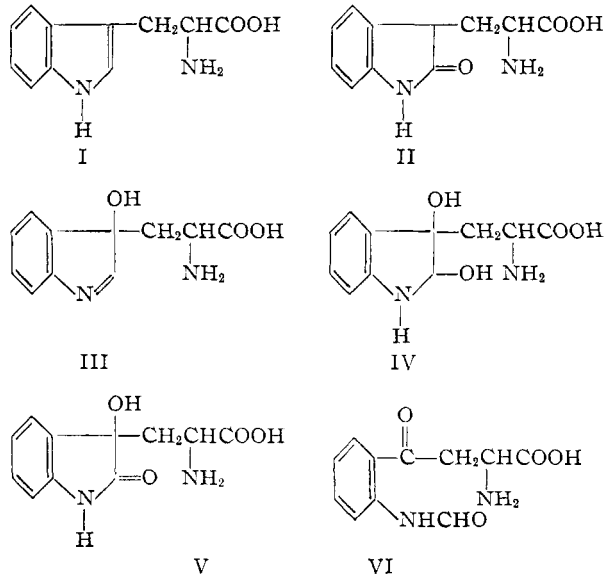
Studies in the Indole Series. XVI.[1] Oxindole-3-alanine and Dioxindole-3-alanine

BY PERCY L. JULIAN, EARL E. DAILEY, HELEN C. PRINTY, HYMAN L. COHEN AND SHINICHI HAMASHIGE[2]

RECEIVED DECEMBER 30, 1955

A new synthesis of oxindole-3-alanine is devised and three distinct improvements and safeguards are suggested for the Cornforth synthesis of this substance, thus making this amino acid a readily available material for further syntheses of substances related to natural products. Dioxindole-3-alanine, suggested as a possible intermediate in the metabolic transformation of tryptophan into formylkynurenine, also has been synthesized. Condensation of the tetrahydropyranyl ether (VII) of dioxindole with methoxymethylmalonic ester, hydrolysis, nitrosylation of the resulting lactone XI of α-carboxy-β-dioxindolyl-3-propionic acid (X) and catalytic hydrogenolysis of the obtained lactone XII of α-oximino-β-dioxindolyl-3-propionic acid (XVII) lead, on the one hand, to oxindole-3-alanine, while catalytic reduction of the oximino acid XVII itself gave dioxindole-3-alanine in two diastereomeric forms, separated and characterized by several derivatives and by use of the Dakin–West reaction.

The search for the missing chemical link, or links, between tryptophan (I) and formylkynurenine (VI) in the metabolic transformation of the former,[1] has been the subject of numerous endeavors over the past two decades. The literature contains several recent summaries[3] of this work. Four potential intermediates have been suggested for this metabolic pathway: oxindole-3-alanine (II), 3-hydroxyindolenine-3-alanine (III), 2,3-dihydroxyindoline-3-alanine (IV) and dioxindole-3-alanine (V). The synthesis of the first of these (II) has recently been reported from six laboratories.[4] It has been eliminated as an intermediate because of its inactivity in the oxidase–peroxidase system of Knox and Mehler,[5] as well as with the tryptophan-



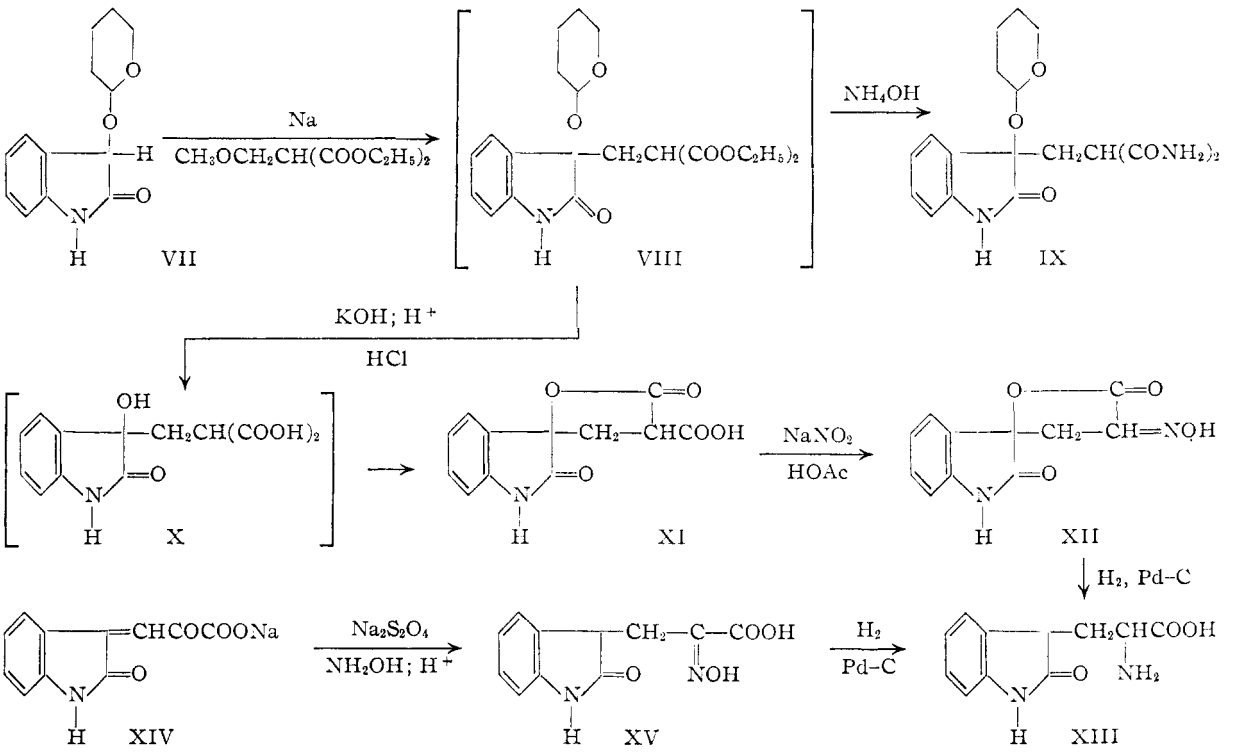
(1) For paper XV in this series, see THIS JOURNAL, **78**, 3501 (1956).

(2) Antioch College Cooperative student, March to August, 1952.

(3) (a) C. E. Dalgliesh, *Quart. Revs. (London)*, **5**, 227 (1951); (b) P. L. Julian, E. W. Meyer and H. C. Printy in Elderfield's "Heterocyclic Compounds," Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1952, p. 63, 182; (c) A. Ek, H. Kissman, J. B. Patrick and B. Witkop, *Experientia*, **8**, 36 (1952); (d) A. H. Mehler in "Amino Acid Metabolism," edited by William McElroy and Bentley Glass, The Johns Hopkins Press, Baltimore, Md., 1955, p. 882.

(4) (a) B. Witkop, *Ann.*, **558**, 98 (1947); (b) M. Kotake, T. Sakan and T. Miwa, THIS JOURNAL, **72**, 5085 (1950); (c) J. W. Cornforth, R. H. Cornforth, C. E. Dalgliesh and A. Neuberger, *Biochem. J.*, **48**, 591 (1951); (d) H. Behringer and H. Weissauer, *Chem. Ber.*, **85**, 743 (1952); (e) H. Rinderknecht, H. Koechlin and C. Niemann, *J. Org. Chem.*, **18**, 971 (1953); (f) T. Wieland, O. Wieberg and W. Dilger, *Ann.*, **592**, 69 (1955).

(5) (a) W. E. Knox and A. H. Mehler, *J. Biol. Chem.*, **187**, 419 (1950); (b) A. H. Mehler and W. E. Knox, *ibid.*, **187**, 431 (1950); (c) C. E. Dalgliesh, W. E. Knox and A. Neuberger, *Nature*, **168**, 20 (1951).



adapted Pseudomonas strain of Hayaishi.[6]

This communication reports the synthesis of another of the suggested intermediates, dioxindole-3-alanine (V). In view of the success attendant upon the Michael condensation of the tetrahydropyranyl ether (VII) of dioxindole with acrylic ester, leading to dioxindole-3-propionic acid,[1] it appeared that a similar condensation of VII with methoxymethylmalonic ester would provide suitable material for the synthesis of the much-desired amino acid, dioxindole-3-alanine (V), and indeed this has been realized.

The sodio derivative of 3-(tetrahydropyran-2-yloxy)-oxindole (VII) was rapidly formed as a benzene-soluble product when VII was added to a suspension of powdered sodium in benzene. Addition of ethyl methoxymethylmalonate, likewise in benzene solution, yielded ethyl α-carboethoxy-β-[3-(tetrahydropyran-2-yloxy)-oxindolyl]-3-propionate (VIII), an oil which did not crystallize, nor would distil without decomposition, but which gave in 60% yield the expected crystalline diamide IX on treatment with concentrated aqueous ammonia.

The crude ester VIII was, therefore, subjected to alkaline hydrolysis with aqueous potassium hydroxide, and the product from cautious acidification immediately extracted with ether. On passing dry hydrogen chloride into the cold ether extract, the crystalline lactone XI of α-carboxy-β-dioxindolyl-3-propionic acid (X) separated. It was appropriately purified and characterized by its analysis and infrared spectrum, which latter showed significantly the absence of the hydroxyl group so dominant in the spectrum of dioxindole-3-propionic acid.

Nitrosylation of the lactone XI with sodium nitrite in glacial acetic acid solution gave the beautifully crystalline lactone XII of α-oximino-β-dioxindolyl-3-propionic acid (XVII). The oximino lactone XII on treatment with concentrated ammonium hydroxide gave the oximino acid amide XIX.

On catalytic hydrogenation of XII over a palladium-on-carbon catalyst in 80% aqueous methanol, somewhat to our surprise we obtained oxindole-3-alanine (XIII) in good yield! Despite our fears that this outcome of hydrogenolysis might spell death to our hopes for obtaining dioxindole-3-alanine (XVIII) by catalytic hydrogenation of α-oximino-β-dioxindolyl-3-propionic acid itself (XVII),[7] this remarkably clean, simple and high-purity preparation of oxindole-3-alanine came as a very pleasant surprise after twenty years of effort to effect a facile preparation of this substance by our traditional alkylation procedures with oxindoles.

In view of difficulties up to the present in devising practical and large-scale syntheses of β-oxindolyl-3-ethylamines for use in synthesizing substances having the yohimbine ring skeleton,[8] the ready availability of the alternative oxindole-3-alanines would indeed be welcome. Because of this we have devoted considerable time to improvements of the only one of the reported oxindole-3-alanine syntheses[4] which lends itself to this purpose, namely, that of Cornforth, *et al.*[4c] Simple in outline, and of wide potential utility because of the availability of substituted isatins, this synthesis as reported offers some major difficulties to the uninitiated worker in

(6) T. Sakan and O. Hayaishi, *J. Biol. Chem.*, **186**, 177 (1950).

(7) Benzyl alcohol is quantitatively converted to toluene by catalytic hydrogenation over a palladium-on-carbon catalyst; W. H. Hartung in Adams, "Organic Reactions," Vol. VII, John Wiley and Sons, Inc., New York, N. Y., p. 263. Dioxindole-3-propionic acid[1] was recovered unchanged on attempted hydrogenolysis over palladium-on-carbon catalyst.

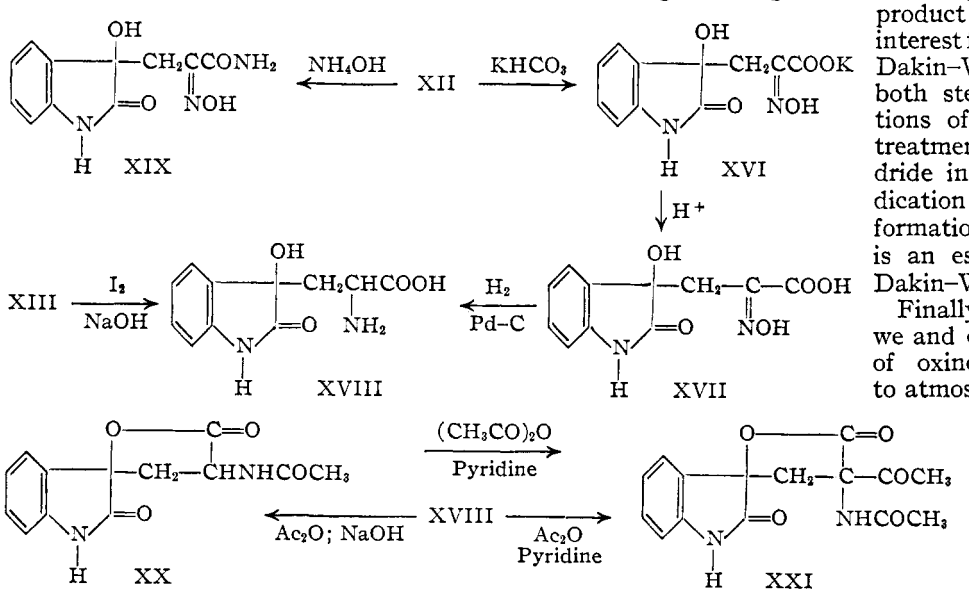
(8) P. L. Julian, A. Magnani, H. C. Printy and E. E. Dailey, unpublished work.

this field. The isatylidenepyruvic acid obtained in the first reaction is nearly always accompanied by tars and in this condition is almost useless for the remaining steps.[9] We have found that preparation of the crystalline sodium salt XIV of this acid obviates this difficulty.

Likewise, in our hands the oximino acid XV was difficult to purify, invariably showing low nitrogen content on analysis. Utilization of the crystalline sodium salt XIV for its preparation, and conversion of the crude oximino acid XV into a pure crystallizate containing one mole of tetrahydrofuran per one mole of XV, corrected this difficulty. The crystalline tetrahydrofuran complex on solution in methanol and addition of water, gave pure oximino acid XV.

A third difficulty with the Cornforth preparation resides in the laborious reduction with sodium amalgam, and the concomitant necessity for removal of large quantities of anions and cations in the purification of the amino acid XIII. We have found that the oximino acid XV in 80% aqueous methanol is converted in 82% yield into almost pure oxindole-3-alanine (m.p. crude, 250–255°) by catalytic hydrogenation over a palladium-on-carbon catalyst.

With the above improvements and safeguards for the Cornforth preparation, oxindole-3-alanine is now a readily available substance by their method or by the method described in this communication.



a from low-melting isomer
b from high-melting isomer

Returning to the major objective, the preparation of dioxindole-3-alanine (XVIII), the oximino lactone XII on treatment with aqueous potassium bicarbonate gave the crystalline potassium salt XVI and this product on acidification gave crystalline α-oximino-β-dioxindolyl-3-propionic acid (XVII). In contradistinction to the oximino lactone XII its infrared spectrum showed the presence of an OH group and the absence of a lactone carbonyl.

On hydrogenation over a palladium-on-carbon catalyst, XVII was converted into two products, both having the structure XVIII. The one of these, m.p. 232° dec., was a hydrate of XVIII which could not be induced to lose its water of crystallization. The other melted at 260° with decomposition, gave analyses corresponding to XVIII, and was not converted into the low-melting hydrate by repeated crystallizations from water.

Proof that the low-melting and high-melting substances are diastereoisomeric modifications of dioxindole-3-alanine (XVIII) and not hydrated and non-hydrated crystalline forms, respectively, of the same stereochemical configuration was proved as follows: Both the low and high melting isomers gave non-hydrated N-acetyl lactone derivatives XX, melting at 284° dec. and 226°, respectively, on treatment with acetic anhydride in aqueous sodium hydroxide solutions. The two isomers also gave different DNP derivatives when treated with 1-fluoro-2,4-dinitrobenzene, the one from the low-melting isomer melting at 170° dec. and that from the high-melting isomer showing no definite melting point but shrinking at 270° with slow decomposition. The Dakin–West reaction with the two diastereomeric dioxindole-3-alanines (XVIII) gave the final proof of their stereoisomerism. Both the high-melting and low-melting forms gave the same product XXI, m.p. 266°. Of interest for the chemistry of the Dakin–West reaction is that both stereoisomeric modifications of XX gave XXI on treatment with acetic anhydride in pyridine, another indication that neither azlactone formation nor decarboxylation is an essential phase of the Dakin–West reaction.[10]

Finally, the sensitivity which we and others[4] have observed of oxindole-3-alanine (XIII) to atmospheric oxygen, led us to attempt its conversion to dioxindole-3-alanine (XVIII) by the iodine–sodium hydroxide technique employed by Kendall for conversion of oxindole-3-propionic acid into dioxindole-3-propionic acid.[11] A substance of m.p. 228° and showing the same characteristic ninhydrin test and infrared spectra as the low melting isomer of dioxindole-3-alanine, was obtained.

This conversion of oxindole-3-alanine (XIII) to dioxindole-3-alanine (XVIII) by a reaction which avoids extensive heating with alkali, suggests the generality of the reaction noted by Kendall and by

(9) In a private communication to Miss Printy, Professor Cornforth writes "The quality of the isatylidenepyruvic acid might be important; it should be wholly crystalline and show no tarry matter on microscopic examination."

(10) *Cf.* (a) G. H. Cleland and C. Niemann, THIS JOURNAL, **71**, 841 (1949); (b) R. H. Wiley and O. H. Borum, *ibid.*, **72**, 1626 (1950).

(11) E. C. Kendall, A. E. Osterburg and B. F. MacKenzie, *ibid.*, **48**, 1384 (1926).

this Laboratory[1] whereby 3-alkyloxindoles are readily oxidized to 3-alkyldioxindoles. At the same time, such a reaction throws grave doubt upon the likelihood that dioxindole-3-alanine will prove to be an intermediate between tryptophan and formylkynurenine in tests where oxindole-3-alanine has shown inactivity. We can hardly conceive that the oxidase–peroxidase systems, used as criteria for this physiological activity,[5,6] would fail to effect rapid conversion of XIII into XVIII, in view of our numerous applications of this mode of conversion to 3-alkyloxindoles. Nevertheless, Drs. Witkop, Mehler and Hayaishi have graciously indicated their eagerness to subject our diastereoisomeric dioxindole-3-alanines to these tests and appropriate samples for this purpose have been sent to Dr. Bernhard Witkop.[11a]

Experimental[12]

Ethyl α-Carboethoxy-β-[3-(tetrahydropyran-2-yloxy)-oxindolyl]-3-propionate (VIII).—Under a nitrogen atmosphere and with agitation 46.7 g. (0.2 mole) of 3-(tetrahydropyran-2-yloxy)-oxindole (VII),[1] dissolved in 300 ml. of dry benzene, was added to 5.06 g. (0.2 mole plus 10% excess) of powdered sodium in 100 ml. of dry benzene. The resulting pale yellow solution was free of solid material after stirring for one hour. During ten minutes 48 g. of ethyl methoxymethylmalonate was then added to the solution, cooled in an ice-bath. The solution was allowed to come to room temperature and stirred for 12–15 hours, during which time it went through a series of color changes. The solution was then washed with ice-water until neutral, and then concentrated in vacuum to a red oil weighing 80 g. Attempts to distil this oil resulted in deep-seated decomposition. Likewise, it could not be induced to crystallize.

α-Carboxamido-β-[3-(tetrahydropyran-2-yloxy)-oxindolyl]-3-propionamide (IX).—To 9 g. of the crude condensation product (VIII) 50 ml. of concentrated ammonium hydroxide was added and the mixture agitated for three days. The red oil was slowly replaced by 4.38 g. (57%) of colorless crystals which melted at 230–233° dec. They were recrystallized first from acetone and then from pyridine, m.p. 244–245° dec.

Anal. Calcd. for $C_{17}H_{21}O_5N_3$: C, 58.77; H, 6.09; N, 12.09. Found: C, 59.12; H, 6.09; N, 11.89.

α-Carboxy-β-dioxindolyl-3-propionic Acid Lactone (XI).—To 80 g. of crude ethyl α-carboethoxy-β-[3-(tetrahydropyran-2-yloxy)-oxindolyl]-3-propionate (VIII) a solution of 44.8 g. of potassium hydroxide in 150 ml. of water was added, and the mixture heated on the steam-cone for 1.5 hours. The alkaline solution, after cooling, was extracted with two 100-ml. portions of alcohol-free ether to remove any neutral products (0.27 g.). The aqueous layer was concentrated under diminished pressure to a volume of 100 ml., cooled in an ice–salt mixture, and acidified to congo red paper with concentrated hydrochloric acid. On extraction five times with a total volume of 1500 ml. of alcohol-free ether, washing once with a small portion of water, drying over anhydrous magnesium sulfate, and removal of the ether under diminished pressure and low temperature, 61.6 g. of a yellow oil (X) was obtained. It could not be induced to crystallize, and was dissolved in 250 ml. of alcohol-free ether and saturated with dry hydrogen chloride. The oil which first separated, crystallized after ten minutes of stirring. Stirring was continued for one hour. The product was filtered, and 20.6 g., m.p. 185° dec., was obtained; yield 42%. Recrystallization from acetone–ether gave colorless powdery crystals, m.p. 188–190° dec.

Anal. Calcd. for $C_{12}H_9O_5N$: C, 58.30; H, 3.66; N, 5.66. Found: C, 58.63; H, 3.81; N, 5.98.

The infrared spectrum showed no hydroxyl band, but a distinct lactone carbonyl band at 5.63 μ. The lactone acid XI was refluxed in water for 2.5 hours and recovered unchanged.

α-Oximino-β-dioxindolyl-3-propionic Acid Lactone (XII).—To a suspension (cooled to 20°) of 2.47 g. of α-carboxy-β-dioxindolyl-3-propionic acid lactone (XI) in 50 ml. of glacial acetic acid, 4.14 g. of sodium nitrite in 6 ml. of water was added, with stirring, over a period of 30 minutes (gas evolution, which colored moist starch–iodide paper, took place during the addition). Solution was complete when approximately one-third of the sodium nitrite had been added. Shortly afterward the oximino lactone XII separated. The mixture was stirred for four hours after the addition of the sodium nitrite solution. The product was filtered 2.55 g., m.p. 185° dec. It contained sodium acetate, and upon recrystallization from aqueous methanol, 1.82 g. (72%), m.p. 215° dec., was obtained. The analytical sample melted at 218° dec. after recrystallization from aqueous methanol.

Anal. Calcd. for $C_{11}H_8O_4N_2$: C, 56.89; H, 3.47; N, 12.06. Found: C, 56.83; H, 3.12; N, 11.72.

It was noticeable that when melted in soft glass tubes (which on powdering and slurrying in water showed distinct alkalinity), the decomposition point of the oximino lactone was always lower than in Pyrex tubes.

Oxindole-3-alanine (XIII) from Reductive Hydrogenolysis of α-Oximino-β-dioxindolyl-3-propionic Acid Lactone (XII).—In 100 ml. of 80% methanol and 6.96 g. of 10% palladium-on-carbon, 6.96 g. (0.03 mole) of XII was hydrogenated at room temperature and atmospheric pressure. The first moles of hydrogen were absorbed very rapidly (24 minutes); four hours, however, were required for absorption of the third mole. In the meantime, oxindole-3-alanine (XIII) had precipitated onto the catalyst. After reduction, the solution was filtered and concentrated *in vacuo* with a nitrogen leak. When a low volume was obtained, absolute alcohol was added and the solution was again concentrated under nitrogen until crystallization began. Thus, 1.5 g. of oxindole-3-alanine (XIII), m.p. 250° dec., was obtained. The catalyst was extracted with 200 ml. of boiling water for 15 minutes, filtered and worked up as aforestated to yield an additional 2.6 g. of oxindole-3-alanine, m.p. 252° dec., yield 60%. The oxindole-3-alanine thus obtained exhibited the same pale violet-blue ninhydrin color of authentic oxindole-3-alanine[4c] and an identical infrared spectrum.

Sodium Isatylidene-3-pyruvate (XIV).—A suspension of 29.2 g. (0.2 mole) of isatin in a mixture of 60 ml. of absolute ethanol and 23.6 g. (0.2 mole) of ethyl pyruvate, was cooled to 5°. With stirring and further cooling, 16.8 g. (0.2 mole) of diethylamine was added dropwise over a period of ten minutes. The temperature rose to 11–13°. Following addition, the mixture was stirred for ten more minutes and then filtered (recovered isatin, 3 g., 10%). The filtrate was again cooled to 5° and a solution of 16.8 g. (0.2 mole) of sodium bicarbonate in 100 ml. of water was added. A granular precipitate separated. After stirring at 5° for 15 minutes, the mixture was filtered, washed with three 25-ml. portions of cold water, then three 25-ml. portions of alcohol and air-dried; yield 25 g. (55%), of brick-red crystalline sodium salt.

The yield was increased to 38 g. (80%) by use of 8.0 g. (0.2 mole) of sodium hydroxide (instead of bicarbonate) in 25 ml. of water and 250 ml. of absolute alcohol added dropwise over a period of an hour. The mixture in this case, however, must be carefully centrifuged, and washed well by centrifugation with water, ethanol and finally with ether, to avoid the retention of strong alkali and subsequent rearrangement to the quinoline-2,4-dicarboxylic acid.

Anal. Calcd. for $C_{11}H_8NO_3Na$: Na (calcd. as Na_2O), 12.85; N, 5.80. Found: Na (as Na_2O), 12.71; N, 5.44.

A suspension of 4.74 g. (0.02 mole) of the sodium salt in 25 ml. of water was cooled to 0° and 10 ml. of 2 *N* hydrochloric acid was added dropwise with stirring. The mixture was cooled, stirred for 15 more minutes, then filtered, washed with three 5-ml. portions of cold water, and air-dried; yield 3.36 g. (80%) of pure isatylidene-3-pyruvic acid,[4c] m.p. 200–205° dec.

(11a) NOTE ADDED IN PROOF.—Dr. Witkop informs us that "in the oxidase–peroxidase system of Knox and Mehler, as well as with *Pseudomonas* strain 6, using either impact cells or extracts, both of your preparations were found to be completely inactive, nor did they cause any inhibition of the normal tryptophan metabolism when the latter was added to the system. The two forms of dioxindolylalanine may safely be excluded as intermediary products in the enzymatic degradation of tryptophan."

(12) All melting points are uncorrected. Analyses by Micro Tech Laboratories, Skokie, Ill.

α-Oximino-β-oxindolyl-3-propionic Acid (XV).—A suspension of 4.8 g. (0.02 mole) of sodium isatylidene-3-pyruvate (XIV) in 20 ml. of water was cooled to 5°, and 3.5 g. (0.02 mole) of sodium dithionite was added portionwise over a period of ten minutes. The mixture was stirred an additional hour. Three grams of Norite was added and the mixture was rapidly brought to a boil and kept there for five minutes. It was then filtered hot and cooled to room temperature. To the amber liquid 13.0 g. (0.2 mole) of hydroxylamine hydrochloride was added; 40 ml. of 2 *N* sodium hydroxide was then added and the solution was allowed to stand for one hour. It was then cooled to 0° and 33 ml. of 2 *N* hydrochloric acid was added dropwise. The oil which initially formed, solidified rapidly to a yellow granular crystal mass. After standing in the refrigerator overnight, it was filtered and air-dried; yield 24 g. (50%), m.p. 163–168°. Recrystallization from aqueous methanol and careful drying *in vacuo* gave a product, m.p. 168–170°, which analyzed for 9.63 nitrogen, instead of the expected 11.96. This had been our experience invariably.

Accordingly 1.6 g. of the oximino acid XV thus obtained was dissolved in 5 ml. of boiling tetrahydrofuran. The mixture was filtered and cooled. On standing overnight 1.2 g. of microcrystals separated. This was again recrystallized from 4 ml. of tetrahydrofuran, yield 0.8 g.

Anal. Calcd. for $C_{15}H_{18}O_6N_2$: C, 58.83; H, 5.92; N, 9.15; mol. wt., 304. Found: C, 58.96; H, 6.00; N, 9.27; mol. wt. (by titration), 306. The product is, therefore, a crystallizate containing one mole of tetrahydrofuran to one mole of oximino acid XV.

To a solution of 500 mg. of the oximino acid–tetrahydrofuran complex in 2 ml. of hot methanol, 2 ml. of water was added. On cooling overnight, 200 mg. of oximino acid XV, m.p. 170–172°, was collected.

Anal. Calcd. for $C_{11}H_{10}O_4N_2$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.20; H, 4.44; N, 11.84.

Oxindole-3-alanine (XIII) from α-Oximino-β-oxindolyl-3-propionic Acid (XV).—A solution of 4.6 g. (0.02 mole) of the oximino acid XV in 250 ml. of 80% (by volume) methanol was hydrogenated over 3 g. of 10% palladium-on-charcoal for three hours at 1.5 atmospheres. The hydrogen consumed was 0.45 mole (110%). The catalyst was filtered and washed twice with 25 ml. of water. The combined filtrate was evaporated to dryness leaving 3.9 g. (82%) of solid melting at 250–255° dec. This was dissolved in 300 ml. of boiling water, the solution concentrated to 10 ml. *in vacuo* with a nitrogen leak, 25 ml. of absolute methanol added, and the mixture refluxed under nitrogen for five minutes, cooled and filtered; yield 2.2 g., m.p. 254–256° dec.

Anal. Calcd. for $C_{11}H_{12}O_3N_2$: C, 59.99; H, 5.50; N, 12.72. Found: C, 60.12; H, 5.40; N, 12.48.

Potassium α-Oximino-β-dioxindolyl-3-propionate (XVI).—A suspension of 11.6 g. (0.05 mole) of α-oximino-β-dioxindolyl-3-propionic acid lactone (XII) in 100 cc. of water containing 5 g. of potassium bicarbonate, was heated on a steam-cone for 10 minutes, after which time solution was complete (evolution of gas). The solution was allowed to cool and cream-yellow plates separated, which were filtered, yield 8.5 g. (56%, based upon hydrated formula for XVI). Recrystallized from water it was obtained as lustrous colorless plates, m.p. 170° dec.

Anal. Calcd. for $C_{11}H_9O_5N_2K$: C, 45.82; H, 3.14; N, 9.71; K, 13.56. Calcd. for $C_{11}H_9O_5N_2K \cdot H_2O$: C, 43.12; H, 3.61; N, 9.14; K, 12.77. Found: C, 43.24; H, 4.08; N, 9.28; K, 13.32 (ash calcd. as K_2CO_3).

α-Oximino-β-dioxindolyl-3-propionamide (XIX).—Two grams of the oximino lactone XII was dissolved in 10 ml. of concentrated ammonium hydroxide at room temperature. The solution, after 30 minutes, was concentrated to about 5 ml. when crystallization began. Water was then added to the mixture and the mixture concentrated to a low volume. This procedure was repeated until the solution was neutral. After cooling and filtering, 1.99 g., m.p. 197° dec., was obtained (93%). It depressed the melting point of the oximino lactone XII. Recrystallized from water, the amide XIX was obtained as pale yellow prisms, m.p. 201°.

Anal. Calcd. for $C_{11}H_{11}O_4N_3$: C, 53.00; H, 4.44; N, 16.86. Found: C, 52.89; H, 4.50; N, 16.18.

α-Oximino-β-dioxindolyl-3-propionic Acid (XVII).—Eight grams of the potassium salt XVI was dissolved in 12 ml. of 2.4 *N* hydrochloric acid, after which the oxime acid XVII crystallized immediately and was cooled and filtered; yield 6.2 g., m.p. 168° dec. It crystallized from methanol–benzene as colorless microcrystals, m.p. 172° dec.

Anal. Calcd. for $C_{11}H_{10}O_5N_2$: C, 52.81; H, 4.00; N, 11.20. Found: C, 52.86; H, 4.21; N, 10.94.

Dioxindole-3-alanine (XVIII) (from XVII in 80% Methanol).—A solution of 3.75 g. (0.015 mole) of oximino acid XVII in 150 ml. of 80% methanol was hydrogenated over 1.25 g. of 10% palladium-on-carbon catalyst at 4 to 5 atmospheres. The catalyst was first prereduced. The reduction proceeded quite slowly, probably owing to the sparing solubility of the amino acid and its separation onto the catalyst. After 18 hours, uptake of hydrogen ceased. The solution was filtered, concentrated *in vacuo* with a nitrogen leak to a volume of approximately 20 ml., 100 ml. of absolute methanol added, and the solution further concentrated until crystallization began. The mixture was cooled and filtered, giving 0.55 g. of dioxindole-3-alanine, m.p. 257° dec. Recrystallized from water, in which it is soluble only at about 200 to 1, the amino acid XVIII was obtained as colorless needles, m.p. 259° dec. The alcoholic mother liquor on concentration and addition of ether gave 2.3 g. of recovered oximino acid XVII. While oxindole-3-alanine exhibits a pale bluish-violet color in the ninhydrin test, dioxindole-3-alanine (XVIII) gives a more intense royal blue, with only an overtone of violet.

Anal. Calcd. for $C_{11}H_{12}O_4N_2$: C, 55.92; H, 5.12; N, 11.86. Found: C, 56.17; H, 5.31; N, 11.72.

On extracting the filtered catalyst above with 300 ml. of hot water (under nitrogen) for 15 minutes, filtering and concentrating, 0.63 g. of an isomeric dioxindole-3-alanine was obtained, m.p. 228° dec. It recrystallized from water as colorless prisms, m.p. 231° dec., and showed the same color in the ninhydrin test as the high-melting isomer described above.

Anal. Calcd. for $C_{11}H_{12}O_4N_2 \cdot H_2O$: C, 51.96; H, 5.55; N, 11.01. Found: C, 51.70; H, 5.46; N, 10.92.

Counting the recovered oximino acid XVII, the yield of the dioxindole-3-alanines was 90% of theory. It was, however, found more feasible for larger runs to carry out the hydrogenation in aqueous solution where the reduction was over in three hours or less. Fractional recrystallization was made possible by the fact that the lower melting hydrate crystallized more readily from the mixture, although their solubilities were almost identical. The yield was 70%.

Dioxindole-3-alanine (from XVII in 80% Methanol-containing Hydrochloric Acid).—A solution of 7.0 g. (0.28 mole) of oximino acid XVII in 200 ml. of 80% methanol and 7 ml. of concentrated hydrochloric acid was hydrogenated over 3 g. of palladium-on-charcoal catalyst. Reduction was complete in 2.5 hours. The catalyst was removed by filtration and the filtrate concentrated to a volume of 50 ml. (under nitrogen). The theoretical amount of sodium acetate was added, whereupon 4.2 g. (62%) of the mixed isomeric dioxindole-3-alanines was obtained. Recrystallization from water at 200:1 yielded first 1.8 g. of material melting at 232° dec. From the mother liquors 1.78 g. of material was obtained, m.p. 248° dec. On recrystallization from water (200:1) the latter material melted at 259° dec.

Attempts to prepare a crystalline hydrochloride from, or to esterify, either of the dioxindole-3-alanines proved unsuccessful.

Infrared Spectra of the Diastereomeric Dioxindole-3-alanines.—The infrared spectra were taken on a Baird double beam infrared recording spectrophotometer and were found to be generally in accord with recent observations[13] on amino acids bearing a hydroxyl function and on diastereoisomeric amino acids. Measurements were made in the solid state, using KBr pellets.

The High Melting Isomer.—Here we see a loss of the <3 μ OH vibration frequency. A broad band appearing at 3.1 μ and which may be attributed to N-H absorption, masks out the usual hydroxyl band. This phenomenon is exhibited in several spectra of amino acids containing hydroxyl functions.[13] The band at 5.75 μ represents the lactam carbonyl and no acid carbonyl band is present. The charac-

(13) (a) W. A. Bolhofer, THIS JOURNAL, **76**, 1322 (1954); (b) R. J. Koegel, J. P. Greenstein, M. Winitz, S. M. Birnbaum and R. A. McCallum, *ibid.*, **77**, 5708 (1955).

teristic amino acid absorption peaks appear at 6.3, 7.1 and 7.3 μ. The bands at 6.6, 6.9 and 7.5 μ are absent.[13]

The Low Melting Isomer.—This isomeric modification differs from the high melting isomer in having a hydroxyl band at 2.8 μ and in addition the broad band at 3.1 μ (compare β-phenylserine and β-allophenylserine[13b]). The lactam carbonyl band is at 5.8 μ. The characteristic amino acid peaks are present at 6.3, 6.6, 7.1, 7.3 and 7.5 μ. The band at 6.9 μ is absent.[13b]

DNP Derivatives of the Dioxindole-3-alanines. α-2,4-Dinitrophenylamino-β-dioxindolyl-3-propionic Acid. (A) From the Low Melting Dioxindole-3-alanine.—To a solution of 0.1 g. of dioxindole-3-alanine, m.p. 232°, in 4 ml. of a 4% solution of sodium carbonate, 0.149 g. (100% molar excess) of 1-fluoro-2,4-dinitrobenzene was added, and the mixture stirred vigorously at 40° for 4 hours. The mixture was extracted with ether to remove unreacted fluorodinitrobenzene and the aqueous extract acidified with dilute hydrochloric acid and let stand overnight. A yellow precipitate of 0.15 g., m.p. 160° dec., of the DNP derivative was obtained. Repeated recrystallization from ether–petroleum ether and finally acetone–ether gave yellow needles, m.p. 170° dec.

Anal. Calcd. for $C_{17}H_{14}O_8N_4$: C, 50.74; H, 3.50; N, 13.92. Found: C, 51.21; H, 3.69; N, 13.55.

(B) From the High Melting Dioxindole-3-alanine.—A similar preparation from the high melting isomer gave a product which showed no definite melting point, shrinking at about 270° and slowly decomposing as the temperature was raised. The analyses, however, after recrystallization from acetone–ether indicated it to be pure DNP derivative.

Anal. Calcd. for $C_{17}H_{14}O_8N_4$: C, 50.74; H, 3.50; N, 13.92. Found: C, 50.75; H, 3.40; N, 13.64.

Acetyl Derivatives of the Dioxindole-3-alanines. (A) α-Acetamino-β-dioxindolyl-3-propionic Acid Lactone (XXa) (from Low Melting XVIII).—To an ice-cold solution of 0.114 g. of dioxindole-3-alanine, m.p. 232°, in 0.53 ml. of 2 *N* sodium hydroxide (in an atmosphere of nitrogen), 0.46 g. of acetic anhydride was added dropwise with swirling. The reaction mixture at first formed two layers which became a solution on swirling and heating to 35°, whereupon colorless platelets began to separate. Heating was continued at 35–45° for four hours. After cooling in an ice-bath and acidifying with 0.12 ml. of 6 *N* sulfuric acid, 0.1 g. of XXa, m.p. 274° dec., was filtered. The ninhydrin test was negative. Several recrystallizations from acetone gave microcrystals, m.p. 284° dec.

Anal. Calcd. for $C_{13}H_{12}O_4N_2$: C, 59.98; H, 4.64; N, 10.76. Found: C, 59.99; H, 4.71; N, 10.71.

(B) α-Acetamino-β-dioxindolyl-3-propionic Acid Lactone (XXb) (from the High Melting XVIII).—A similar preparation to that described in "A" above yielded from dioxindole-3-alanine, m.p. 259°, a lactone amide XXb melting at 226° (no decomposition). The ninhydrin test was negative. It was recrystallized from acetone.

Anal. Calcd. for $C_{13}H_{12}O_4N_2$: C, 59.98; H, 4.64; N, 10.76. Found: C, 59.72; H, 4.62; N, 10.82.

The Dakin–West Reaction with the Isomeric Dioxindole-3-alanines. (A) α-Acetamino-α-aceto-β-dioxindolyl-3-propionic Acid Lactone (XXI). From Low Melting Dioxindole-3-alanine.—A mixture of 0.1 g. of dioxindole-3-alanine, m.p. 232° dec., 0.2 g. of pyridine and 0.35 g. of acetic anhydride was heated on a steam-bath and in a nitrogen atmosphere for five hours. Solution occurred on heating, and after one hour a precipitate formed. The solution was cooled, water carefully added, and the precipitate filtered. Several recrystallizations from acetone gave 0.084 g. of product XXI melting at 265–266° dec.

Anal. Calcd. for $C_{15}H_{14}O_5N_2$: C, 59.59; H, 4.66; N, 9.26. Found: C, 60.16; H, 4.96; N, 9.22.

(B) XXI from High Melting Dioxindole-3-alanine.—A similar preparation from dioxindole-3-alanine, m.p. 259°, gave a product identical with that secured at "A" above in melting point and infrared spectra.

Dioxindole-3-alanine (XVIII) from Oxidation of Oxindole-3-alanine (XIII).—To 1.10 g. (0.005 mole) of oxindole-3-alanine (XIII) dissolved in 5 ml. of 1 *N* sodium hydroxide, cooled to 10°, two equivalents of finely ground iodine was added (1.27 g.). The solution was cooled to 0° and 3 ml. of 5 *N* sodium hydroxide was introduced. After warming to room temperature, 4 ml. of 5 *N* sulfuric acid was added, followed by 0.36 g. of iodic acid in 5 ml. of water. The mixture was warmed gently to coagulate the iodine, filtered, and the filtrate concentrated *in vacuo*. The crystals, m.p. 225° dec., which separated were filtered and recrystallized from water in the form of colorless prisms, m.p. 228° dec. This product was identical in all respects to the low melting dioxindole-3-alanine already described. Its infrared curve was identical with that of the low melting isomer and showed none of the characteristically different bands displayed by the high melting diamer.

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[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Studies on Condensed Pyrimidine Systems. XVII. Some Halogenopurines

BY GERTRUDE B. ELION AND GEORGE H. HITCHINGS

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The syntheses of 2,6-dichloropurine and 2,6-dibromopurine from xanthine and of 6-bromopurine from hypoxanthine are reported. The chlorination of xanthine is favored by the addition of half a molecular equivalent of water to the phosphoryl chloride. Treatment of 6-chloropurine and 2,6-dichloropurine with hydriodic acid leads to 6-iodopurine and a monochloro-monoiodopurine, respectively.

The importance of halogenopurines as intermediates for the synthesis of a variety of substituted purines has been amply demonstrated by Fischer's classical syntheses of the naturally occurring purines from 2,6,8-trichloropurine.[1] In addition, the halogenopurines are of interest in themselves as possible antagonists of the purine moieties of the nucleic acids, which have been actively sought by these laboratories for a number of years.[2–4] Consequently, the synthesis of some new 6-monosubstituted and 2,6-disubstituted halogenopurines was undertaken (Reaction Scheme).

The chlorination of methylated xanthines and of uric acid with phosphoryl chloride or with mixtures of phosphoryl chloride and phosphorus pentachloride has been known for a long time,[5–7] but xanthine itself has not been successfully chlorinated. The attempted chlorination of xanthine with phosphoryl chloride and trimethylamine led only to 2,6-bis-(dimethylamino)-purine.[8] The addition of di-

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(5) E. Fischer, *Ber.*, **28**, 2480 (1895).

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