Anal. Calcd. for $C_{17}H_{13}NO_5$: C, 65.59; H, 4.18; N, 4.50. Found: C, 65.21; H, 4.00; N, 4.30.

It is soluble in acetic acid, chloroform, and acetone. It is insoluble in dilute mineral acids. It gives a greenish-blue fluorescence with concentrated sulfuric acid.

The deacetylation of these flavonols was not successful either with sulfuric acid or with anhydrous aluminum chloride.

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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]

Preparation of 5-Hydroxy-L- and D-Tryptophan[1]

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5-Hydroxy-DL-tryptophan has been resolved by fractional crystallization of the quinine salts of N-carbobenzoxy-5-benzyloxy-DL-tryptophan. Configurations have been assigned to the resolved isomers, and 5-hydroxy-L- and D-tryptophan have been obtained by catalytic hydrogenation of N-carbobenzoxy-5-benzyloxy-L- and D-tryptophan, respectively.

Considerable interest has developed in the metabolism of 5-hydroxyindole compounds following the identification of serotonin[2] and enteramine[3] as 5-hydroxytryptamine (5HTA). DL-Tryptophan labelled with carbon-14 has been shown to be converted to 5-hydroxytryptophan (5HT) in the salivary glands of the toad *Bufo Marinus*,[4] tryptophan is converted to 5HT in *Chromobacterium violaceum*[5,6] and a specific decarboxylase which converts 5HT to 5HTA is present in the kidney tissue of dogs and guinea pigs.[7] Thus, 5-hydroxytryptophan appears to be a naturally occurring amino acid of considerable physiological importance.

Significant differences have been reported in the metabolism of the D- and the L- forms of many amino acids *in vivo*, particularly the aromatic amino acids. The administration of DL-phenylalanine leads to the excretion of phenylpyruvic acid,[8] whereas L-phenylalanine appears to be completely metabolized by infants.[9] D-Tryptophan is to some extent excreted unchanged[10] and is in part converted to indolelactic and indoleacetic acids,[11] whereas L-tryptophan is more completely metabolized by the human.[12] L-DOPA is converted in large part to homoprotocatechuic and homovanillic acids whereas D-DOPA is in part excreted unchanged and a considerable portion of the amount administered remains unaccounted for in the human.[13] Because of the natural occurrence of 5-hydroxytryptophan and because of the desirability of conducting both *in vivo* metabolic experiments and *in vitro* enzymatic studies with pure optical isomers, the resolution of this amino acid was undertaken.

Preliminary attempts to resolve 5-hydroxytryptophan as the N-formyl derivative were unsuccessful. The N-acetyl derivative was considered even less suitable because of difficulties encountered in hydrolyzing the compound in preliminary studies. N-Carbobenzoxy-5-benzyloxy-DL-tryptophan was then prepared and was resolved by fractional crystallization of the quinine salts from a benzene solution. Catalytic hydrogenation of the N-carbobenzoxy-5-benzyloxy-D- and L-tryptophans afforded 5-hydroxy-D-tryptophan and 5-hydroxy-L-tryptophan, respectively. The yield of 5-hydroxy-L-tryptophan from N-carbobenzoxy-5-benzyloxy-DL-tryptophan was 42%. Configuration of the respective antipodes was established by comparison of the rotation of the resolved isomers with those of tryptophan, by the shift in optical rotation of the L- isomer to a more positive value in acid solution[14] and by the papain-catalyzed formation of N-carbobenzoxy-5-benzyloxy-L-tryptophan anilide.[15]

EXPERIMENTAL

N-Carbobenzoxy-5-benzyloxy-DL-tryptophan. 5-Benzyloxy-DL-tryptophan[16,17] (20.0 g., 0.064 mole) was suspended in a

(1) This work was supported by research grants from the National Institutes of Health, United States Public Health Service.

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(17) We wish to express our appreciation to the Upjohn Company for the gift of a generous supply of 5-benzyloxyindole.

solution of 2.60 g. of NaOH in 450 ml. of water and cooled to 0° in a freezing mixture. In five equal portions, over a period of 80 min., was added 12.2 ml. (0.072 mole) of carbobenzoxy chloride and 72 ml. of 1*N* sodium hydroxide (temperature was maintained at −5° and *p*H at 10); the mixture was shaken thoroughly after each addition. The resulting solution was maintained at 0° for 30 min. and was then allowed to warm to room temperature slowly. The solution was extracted once with ether, and the aqueous phase was acidified to *p*H 1.5 with HCl and was extracted with three 250-ml. portions of EtOAc. The combined EtOAc extracts were dried over anhydrous Na_2SO_4 at 5°, treated with charcoal, filtered, and the solvent was removed *in vacuo*. The resulting oil, which partially crystallized, was dissolved in 650 ml. of boiling benzene, and the solution was filtered and cooled to 10° for 30 min. The product was collected, washed with cold benzene, and dried; 23.2 g. (81%), m.p. 132–133°.[18] For analysis, a portion was recrystallized from benzene, m.p. 133–134°.

Anal.[19] Calcd. for $C_{26}H_{24}N_2O_5$: C, 70.25; H, 5.44; N, 6.30. Found: C, 71.09; H, 5.33; N, 6.16.

*Resolution of N-Carbobenzoxy-5-benzyloxy-*DL*-tryptophan.* Carbobenzoxy-5-benzyloxy-DL-tryptophan (47.5 g.) and anhydrous quinine (34.8 g.) were dissolved in 750 ml. of boiling benzene. To promote crystallization, the solution was heated to boiling and cooled three times during a period of 1 hr. The suspension was allowed to stand overnight at room temperature and the crystalline precipitate was collected on a filter, washed with a small volume of benzene and dried *in vacuo;* crop A, 44.4 g., m.p. 136–138°. Crop A was slurried with 500 ml. of benzene, simmered for several minutes and filtered while hot to yield 37.9 g. of white crystalline solid, m.p. 138–140°. The filtrate was cooled to room temperature and yielded an additional 2.0 g., m.p. 137–138°. The two fractions were combined to form crop B, $[\alpha]_D^{22}$ −83.9° (c 1, abs. EtOH). Crop B was simmered with 500 ml. of benzene for 30 min. and filtered to yield crop C; 37.3 g., m.p. 141–142°, $[\alpha]_D^{22}$ −85.8 (c 1, abs. EtOH). Crop C was dissolved in a boiling mixture of 2 l. of benzene and 400 ml. of absolute methanol, filtered, and concentrated *in vacuo* until crystallization commenced. The solution was allowed to stand overnight at room temperature, and was cooled to 10° for 30 min. and filtered to yield 33.8 g. of crop D; $[\alpha]_D^{22}$ −86.9° (c 1, abs. EtOH). Crop D was recrystallized in a similar manner from a boiling mixture of 3 l. of benzene and 60 ml. of abs. EtOH to yield crop E; 30.7 g., m.p. 143–144°, $[\alpha]_D^{22}$ −91.1° (c 1, abs. EtOH). Recrystallization of crop E from a boiling mixture of 4 l. of benzene and 30 ml. of abs. EtOH by the same procedure gave 28.3 g. of crop F; m.p. 168–169° (marked shrinkage at 145°), $[\alpha]_D^{22}$ −92.1 (c 1, abs. EtOH). Recrystallization of crop F in the same manner gave 22.5 g. of crop G, m.p. 145–146°, $[\alpha]_D^{22}$ −94.3° (c 1, abs. EtOH). Further recrystallization did not lead to any further change in the rotation of the salt.

Crop G was suspended in a mixture of 250 ml. of water and 150 ml. of ethyl acetate, and 6*N* HCl was added to the stirred mixture until the *p*H of the aqueous phase was 1.5. The EtOAc was separated and the aqueous phase was extracted two more times with 100 ml. portions of ethyl acetate. The combined ethyl acetate extracts were washed with several small portions of dilute HCl (*p*H 1.5) to remove the last traces of quinine. The extracts were dried over anhydrous Na_2SO_4, treated with charcoal, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in a minimum volume of hot benzene, left at room temperature overnight, and then cooled to 10° for 1 hr. The crystalline product was collected on a filter and dried *in vacuo;* 12.6 g. of N-carbobenzoxy-5-benzyloxy-D-tryptophan (97% yield from salt), m.p. 97–99°, $[\alpha]_D^{22}$ +10.3° (c 1, abs. EtOH). For analysis, a portion was recrystallized three times from hot benzene; m.p. 98–99°.

Anal. Calcd. for $C_{26}H_{24}N_2O_5$: C, 70.25; H, 5.44; N, 6.30. Found: C, 70.28; H, 5.32; N, 6.05.

When the filtrate from crop A was heated to boiling and cooled, further crystallization occurred; 18.8 g. (m.p. 157–158°) of salt was collected. This procedure was repeated on the filtrate and another 11.2 g. (m.p. 156–157°) was obtained. These two fractions were combined as crop H. Crop H was combined with the filtrate from crop B and dissolved in a total volume of 4.75 l. of boiling benzene. The solution was left overnight at room temperature, cooled to 10° for several hours and the crystals which had formed were collected on a filter, washed with cold benzene, and dried *in vacuo;* 7.6 g. of quinine salt was obtained, m.p. 159–160°. The filtrate was concentrated to a volume of 3 l. and a further 20.9 g. of quinine salt, m.p. 157–158°, was thus obtained. These two fractions were combined to form crop I; $[\alpha]_D^{22}$ −35.1° (c 1, abs. EtOH). Crop I was dissolved in 3.6 l. of boiling benzene and crop J was collected after 24 hr. at room temperature; 17.7 g., m.p. 158–159°, $[\alpha]_D^{22}$ −33.3° (c 1, abs. EtOH). Another crop (K) was collected from the same solution after another 24 hr.; 3.0 g., m.p. 157–158°, $[\alpha]_D^{22}$ −32.5° (c 1, abs. EtOH). Crop J was recrystallized from 2.25 l. of boiling benzene to yield 13.7 g. of crop L (m.p. 160–161°), $[\alpha]_D^{22}$ −32.9° (c 1, abs. EtOH), which was combined with crop K and again recrystallized to yield 14.9 g. of crop M; m.p. 160°, $[\alpha]_D^{22}$ −32.4° (c 1, abs. EtOH). Further recrystallization did not lead to a change in the rotation of the salt. Quinine was removed in the manner described for the other isomer; 8.4 g. of *N*-carbobenzoxy-5-benzyloxy-L-tryptophan (98% yield from salt), m.p. 97–99°, $[\alpha]_D^{22}$ −10.1° (c 1, abs. EtOH). For analysis, a small sample was recrystallized from benzene; m.p. 97–99°.

Anal. Calcd. for $C_{26}H_{24}N_2O_5$: C, 70.25; H, 5.44; N, 6.30. Found: C, 70.11; H, 5.33; N, 6.35.

The filtrates from crops I–M were combined and concentrated to a small volume, treated with charcoal, filtered, and fractionated as described above, to provide additional quinine salt from which 4.3 g. of N-carbobenzoxy-5-benzyloxy-L-tryptophan was obtained (total yield, 12.7 g.).

*N-Carbobenzoxy-5-benzyloxy-*L*-tryptophan anilide.* To a mixture of 30 mg. of N-carbobenzoxy-5-benzyloxy-L-tryptophan, 0.6 ml. of 0.05 *M* 2,3-dimercaptopropanol, 5 ml. of 0.1*M* *p*H 5.2 citrate buffer, and 0.13 ml. of aniline was added 4 mg. of crystalline mercuripapain[20] and the mixture was incubated at 39° for 3 days. The reaction mixture was filtered and the product was washed through the filter with small portions of 0.1*N* hydrochloric acid. The combined filtrate and acid washes were adjusted to *p*H 11.5 and extracted with ether for 20 hr. in a continuous extractor. The ether extract was concentrated to dryness and the residual solid was recrystallized from abs. EtOH to yield 16 mg. of white needles, m.p. 187°.

Anal. Calcd. for $C_{32}H_{29}N_3O_4$: N, 8.09. Found: N, 7.76.

The aqueous phase from the ether extraction was acidified to *p*H 1.5 and was extracted with ethyl acetate. No N-carbobenzoxy-5-benzyloxy-L-tryptophan could be recovered.

N-Carbobenzoxy-5-benzyloxy-D-tryptophan (30 mg.) was treated by the same procedure. No anilide was obtained but 18 mg. of N-carbobenzoxy-5-benzyloxy-D-tryptophan (m.p. 98–99°) was recovered.

*5-Hydroxy-*L*-tryptophan.* Palladium oxide (300 mg.) was added to a solution of 2.0 g. of N-carbobenzoxy-5-benzyloxy-L-tryptophan in 150 ml. of abs. EtOH and 1 ml. of water, and hydrogen was bubbled through the mixture for 3 hr. The precipitate which formed was dissolved by the addition of hot water and the solution was filtered under nitrogen. The filtrate was concentrated to dryness *in vacuo* under nitrogen and washed with ethyl acetate to remove

(18) Melting points were taken in open capillary tubes and are uncorrected.

(19) Analyses were performed by the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

(20) We are indebted to Drs. J. R. Kimmel and E. L. Smith of this Laboratory for the mercuripapain used in this procedure.

starting material. The remaining solid was dissolved in a minimium volume (about 4 ml.) of hot water under nitrogen, treated with charcoal, filtered under nitrogen, and allowed to crystallize at 5°. 5-Hydroxy-L-tryptophan was recovered as pale pink needles; 0.55 g., m.p. 273° dec., $[\alpha]_D^{22}$ −32.5° (c 1, water), $[\alpha]_D^{22}$ +16.0° (c 1, 4*N* HCl) (L-tryptophan, $[\alpha]_D^{23}$ −31.5° (c 1, water).[21] The filtrate was concentrated to yield an additional 0.11 g. In this manner, a total of 4.7 g. of 5-hydroxy-L-tryptophan (80% theor.) was obtained from 12.0 g. of N-carbobenzoxy-5-benzyloxy-L-tryptophan.

A sample was prepared for analysis by recrystallization from water under nitrogen.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.65; H, 5.44; N, 12.50.

5-Hydroxy-D-tryptophan. N-Carbobenzoxy-5-benzyloxy-D-tryptophan (2.0 g.) was reduced by the procedure described for the L-isomer to yield 0.60 g. (61% yield) of 5-hydroxy-D-tryptophan; m.p. 274° dec., $[\alpha]_D^{22}$ +32.2° (c 1, water).

A sample was prepared for analysis by recrystallization from water under nitrogen.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.86; H, 5.62; N, 12.50.

5-Hydroxy-L-tryptophan picrolonate. 5-Hydroxy-L-tryptophan (30 mg.) and 36 mg. of picrolonic acid were dissolved in 3 ml. of hot water under nitrogen. The yellow needles which formed on cooling were collected on a filter; 54 mg. (82% yield), m.p. 184–186° (dec.). For analysis, a portion was recrystallized three times from hot water, m.p. 184–186° (dec.).

Anal. Calcd. for $C_{21}H_{20}N_6O_8 \cdot H_2O$: C, 50.19; H, 4.41; N, 16.74. Found: C, 50.45; H, 4.57; N, 17.00.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES DIVISION, NATIONAL DAIRY PRODUCTS CORPORATION]

Potential Antimicrobial Agents. I. Alkyl 4-Oxo-2-alkenoates

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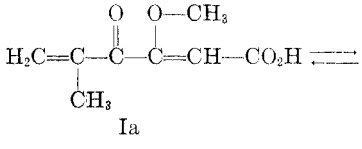
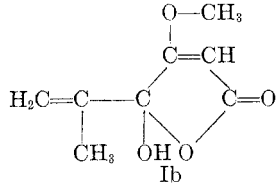
Alkyl 4-oxo-2-alkenoates were conveniently obtained by the retrogressive Diels-Alder reaction of their cyclopentadiene adducts. The requisite adducts were prepared through the interaction of alkylzinc chlorides and half ester chlorides of bicyclo-[2.2.1]5-heptene-2,3-dicarboxylic acid. The analogous reactions were also effected with anthracene adducts.

The structural elucidation of the antibiotic, penicillic acid (Ia, Ib)[1] and its synthesis[2] have

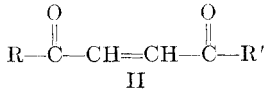


Ia



Ib

stimulated considerable interest in the antimicrobial activity associated with related classes of compounds, notably 4-oxo-2-alkenoic acids (IIa) and certain of their derivatives (IIb, c)



II

R = alkyl, aryl
IIa: R′ = OH
IIb: R′ = alkoxy
IIc: R′ = NH_2, NHR

This interest has focused mainly on β-aroylacrylic acids (IIa)[3a] and their derivatives, esters (IIb)[3b] and amides (IIc),[3c] since the availability of relatively convenient preparative methods in this area was conducive to their examination.

In pronounced contrast there is a lack of adequate preparative methods for the corresponding *aliphatic* analogs of penicillic acid, 4-oxo-2-alkenoic acids, and their derivatives. As a result the antimicrobial properties of but a few compounds of this type have been investigated. Esters of β-acetylacrylic acid[3b,4] and ethyl 4-oxo-2-hexenoate[5] have been shown to have good *in vitro* activity against a number of microorganisms.

Preparatively the esters of β-acetylacrylic acid constitute a special case due to their ready availability from levulinic acid.[6] Ethyl 4-oxo-2-hexe-

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