Water was added, the solution was extracted with ether, and the extract was washed with 2 *N* sulfuric acid and 2 *N* sodium carbonate. The product was chromatographed on alumina (1.5 g.). The major portion crystallized from ether–hexane (20 mg.) to give the (+)-diacetoxydione XXI, m.p. 162–163°; infrared absorption in $CHCl_3$: 1724 cm.$^{-1}$ for ester carbonyl, 1706 cm.$^{-1}$ for ketone carbonyl. For analysis a sample was recrystallized from ether–hexane and dried for 14 hr. at 0.06 mm. (60°), m.p. 163–164°, $[\alpha]^{24}D$ +34.5° (*c* 1.01 in $CHCl_3$).

Anal. Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50. Found: C, 72.25; H, 9.45.

(−)-Hydrogen Phthalate (−)-VIIa, R = CH_3.—The crude acetone-soluble strychnine salt (788 mg.) was dissolved in ethyl acetate (300 ml.) and, in order to regenerate the (−)-hydrogen phthalate, was washed with 10% hydrochloric acid (four portions of 50 ml. each). The crude product (377 mg.), $[\alpha]^{24}D$ −14.5° (*c* 1.08 in $CHCl_3$), contained besides the (−)-hydrogen phthalate some of the (+)-hydrogen phthalate. Recrystallization from methylene chloride–benzene separated the (+)-form as the racemate (120 mg.), m.p. 209–210°, and the mother liquors, when recrystallized from acetone–hexane yielded the pure (−)-hydrogen phthalate as needles (210 mg.), m.p. 187–189°. A sample was recrystallized for analysis twice from acetone–hexane, and dried for 14 hr. at 0.08 mm. (60°); m.p. 188–190°, $[\alpha]^{24}D$ −23.5° (*c* 1.10 in $CHCl_3$).

Anal. Calcd. for $C_{24}H_{30}O_7$: C, 66.96; H, 7.02. Found: C, 66.89; H, 6.93.

(−)-Hydroxyketoacid (−)-VII, R = R′ = H.—A solution of the (−)-hydrogen phthalate (200 mg.) in methanol (50 ml.) was refluxed for 2 hr. with a solution of potassium hydroxide (5 g.), in water (3 ml.). Water was then added, the methanol was stripped off *in vacuo*, and the remaining solution was acidified with sulfuric acid and extracted with ethyl acetate. The crystalline crude product (190 mg.) containing both phthalic acid and the (−)-hydroxyketoacid VII, R = R′ = H, was crystallized from acetone–chloroform to separate off the phthalic acid, m.p. 201–202°. Crystallization of the residue from the mother liquors from acetone–benzene yielded the pure (−)-hydroxyketoacid (51 mg.)., m.p. 171–172°. For analysis a sample was dried for 14 hr. at 0.06 mm. (60°); $[\alpha]^{24}D$ −57.8° (*c* 1.11 in $CHCl_3$).

Anal. Calcd. for $C_{15}H_{24}O_4$: C, 67.13; H, 9.02. Found: C, 67.22; H, 9.15.

(−)-Diacetoxydione VIII from the Acid (−)-VII, R = R′ = H.—A solution of the (−)-acid (−)-VII, R = R′ = H (37 mg.), in methanol (2.5 ml.) containing 0.05% sodium methoxide, was electrolyzed like the (+)-acid VII, R = R′ = H. The methanol was distilled off *in vacuo* and the residue was taken up in a mixture of methylene chloride–ether and washed with 5% sodium carbonate solution. The residue (33 mg.) after evaporation of the solvent was treated with pyridine (0.5 ml.) and acetic anhydride (0.5 ml.) at room temperature overnight. The reaction mixture was worked up as usual. The crude product was chromatographed on alumina (1.0 g.), and the major fractions were crystallized from ether–hexane to give the pure (−)-diacetoxydiketone VIII, R = Ac (11 mg.)., m.p. 165–166°, $[\alpha]^{24}D$ −33.9° (*c* 1.10 in $CHCl_3$), identical in all respects (infrared absorption spectrum, mixture melting point) with the diacetoxydiketone obtained by degradation from natural α-onocerin diacetate. A sample was dried for analysis for 14 hr. at 0.06 mm. (60°).

Anal. Calcd. for $C_{32}H_{50}O_8$: C, 72.41; H, 9.50. Found: C, 72.31; H, 9.48.

Diacetoxy-di-α,β-unsaturated Diester XXII, R = Ac, R′ = Et.—A solution of ethyl bromide (freshly distilled, 290 mg.) in absolute ether (5 ml.) was added under stirring to magnesium (58 mg.) in ether (3 ml.) and the stirring was continued until all the magnesium had disappeared. A solution of ethoxyacetylene (200 mg., prepared according to *Org. Syntheses*, **34**, 46 (1954)) in ether (5 ml.) was now added, and the reaction mixture was boiled for 2.5 hr., during which a brown oil separated. A solution of the (−)-diacetoxydiketone XXII (100 mg.) in ether (10 ml.) was added dropwise, and the mixture was boiled for 2 hr. and stirred overnight until the brown precipitate had disappeared completely. The mixture was then cooled to 0°, poured into 20 ml. of an ice-cooled saturated solution of ammonium chloride, and extracted with ether. The crude product (110 mg.) showed in the infrared absorption bands at 2283 cm.$^{-1}$ for an acetylene bond and at 1724 cm.$^{-1}$ for the acetate functions. It was dissolved in methanol (30 ml.), treated with 10% sulfuric acid (3 ml.), and the mixture was then shaken for 2 hr. to effect rearrangement. Water (50 ml.) was then added, the mixture was extracted with ether, and the ether was washed with sodium bicarbonate solution. The product (110 mg.) was chromatographed on alumina (3 g.) and the benzene fractions (41 mg.) crystallized from methanol to yield the pure di-α,β-unsaturated ester XXII, R = Ac, R′ = Et (35 mg.), m.p. 154–155°. For analysis a sample was dried for 14 hr. at 0.07 mm. (60°); infrared absorption in $CHCl_3$: 1721 cm.$^{-1}$ for acetate carbonyls, 1706 cm.$^{-1}$ for α,β-unsaturated ester carbonyls, and 1642 cm.$^{-1}$ for ethylene bonds; ultraviolet absorption: λ_{max}^{EtOH} 224 mμ, log ε 4.60.

Anal. Calcd. for $C_{40}H_{62}O_8$: C, 71.61; H, 9.32. Found: C, 71.85; H, 9.23.

α-Onocerin Diacetate.—To a solution of the di-α,β-unsaturated ester XXII (25 mg.) in methanol (2 ml.) was added 8 ml. of a 10% solution of potassium hydroxide in 75% aqueous methanol and the mixture was refluxed for 2 hr. Water was added, the mixture was extracted with ether, and the aqueous solution was acidified with dilute sulfuric acid and extracted again with methylene chloride. The crude diacid (22 mg.), obtained from the methylene chloride extract, was dissolved in quinoline (2 ml., freshly distilled), copper–chromite catalyst (20 mg.) was added, and the mixture was refluxed for 1 hr. Ether was then added, and the ether solution was washed with dilute sulfuric acid and then with 10% sodium hydroxide. The residue (20 mg.) was dissolved in pyridine (0.5 ml.) and acetic anhydride (0.5 ml.) and left at room temperature overnight. Ice-water was added, the solution was extracted with ether, and the ether extract was washed with dilute sulfuric acid and then with 10% sodium carbonate. The residue (20 mg.) was chromatographed on alumina (1 g.). The main crystalline fraction (15 mg.) was sublimed under high vacuum (0.08 mm.) for analysis. The sample (12 mg.), m.p. 220–221°, $[\alpha]^{24}D$ + 29.8° (*c* 0.69 in $CHCl_3$), was identical in all respects (infrared absorption spectra, mixture melting point) with an authentic specimen of natural α-onocerin diacetate.[2]

Anal. Calcd. for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.70; H, 10.39.

Acknowledgment.—This work was supported, in part, by grants from the National Science Foundation and the National Institutes of Health.

[CONTRIBUTION FROM THE MEDICAL RESEARCH LABORATORIES, CHAS. PFIZER & CO., INC., GROTON, CONN.]

Chemistry of Indolmycin[1]

BY M. SCHACH VON WITTENAU AND HANS ELS

RECEIVED MAY 29, 1963

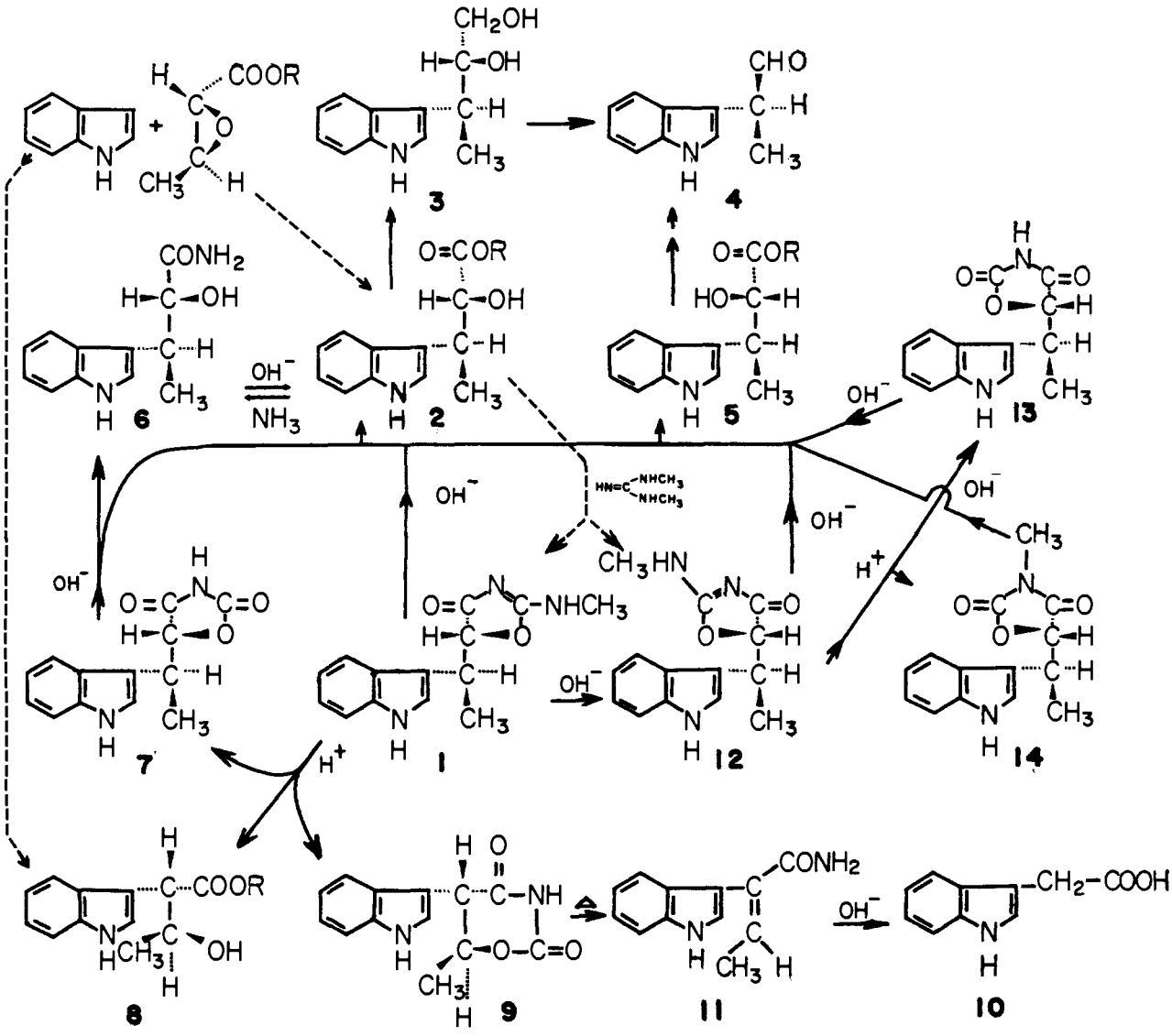
Indolmycin, an antibiotic elaborated by *Streptomyces albus*, was shown to be 2-methylamino-5-α-(β-indolyl)-ethyl-2-oxazolin-4-one (1). Its stereochemistry was deduced from acid hydrolysis products, some of which resulted from 1–2 migration of the indolyl moiety. The epimeric isoindolmycin (12) did not rearrange in this manner. Indolmycin was reconstituted from the α-hydroxy acid **2**, which in turn was prepared in its racemic form by stereospecific synthesis from indole and ethyl 2,3-epoxybutyrate.

The fermentation broth of *Streptomyces albus* strain BA-3972, isolated from an African soil, exhibits antimicrobial activity against strains of staphylococci resistant to commercially available antibiotics.[2] Rao first isolated and characterized the major active component which had been designated PA-155A.[3] Subsequently we have referred to this compound as indolmycin. This paper describes studies concerned with the structure elucidation as well as the synthesis of this antibiotic.

(1) Preliminary communication, M. Schach von Wittenau and H. Els, *J. Am. Chem. Soc.*, **83**, 4678 (1961).

(2) W. S. Marsh, A. L. Garretson, and E. M. Wesel, *Antibiot. Chemotherapy*, **10**, 316 (1960).

(3) K. V. Rao, *ibid.*, **10**, 312 (1960).



Indolmycin (1)[4,5] melts at 213°, has the molecular formula $C_{14}H_{15}N_3O_2$, and possesses one C-methyl group. It shows an ultraviolet absorption spectrum closely resembling that of tryptophan and with Ehrlich reagent gives the color characteristic of α-unsubstituted indoles. The infrared absorption spectrum has a band of medium intensity at 5.82 μ. Indolmycin is a weak base. A titration in glacial acetic acid with perchloric acid showed a neutral equivalent of 253.

Degradation Studies.—Alkaline hydrolysis of indolmycin (1) yielded ammonia, methylamine, carbon dioxide, and two carboxylic acids, α- and β-indolmycenic acids. Both acids have a molecular formula $C_{12}H_{13}NO_3$ and exhibit the characteristic ultraviolet absorption spectra of β-substituted indoles. The α-acid, $[\alpha]^{25}D$ −10°, representing about two-thirds of the mixture, has a melting point of 181–182°, while the β-acid, $[\alpha]^{25}D$ −36°, melts at 142–143.5°.

The presence in α-indolmycenic acid (2, R = H) of an indole moiety unsubstituted in the benzene ring was proved by formation of a small quantity of indole on pyrolysis. Also, oxidation of the α-acid with permanganate furnished anthranilic acid. The color test with Ehrlich reagent indicated the α-position of the indole nucleus to be unsubstituted. This conclusion is supported by the infrared absorption spectrum which shows a band of medium to strong intensity at 9.17 but no bands at 12.8, 7.09 and only a very weak band at 6.45 μ, a pattern characteristic of 3-substituted indoles.[6] α-Indolmycenic acid (2, R = H) therefore consists of an indole with a (C_3H_3O)–COOH side chain attached to the 3-position.

Analysis of the α-acid 2 (R = H) showed one C-methyl group. Further details were obtained from the n.m.r. spectrum of the ethyl ester 2 (R = C_2H_5) which revealed the CH_3–C(H)–C(H)–OH moiety by a doublet (8.7 τ, 3 protons), a multiplet (6.4 τ, 1 proton), a doublet (5.56 τ, 1 proton), and an unsharp peak (6.96 τ, 1 proton). The secondary alcoholic function was also indicated by a peak at 8.95 μ in the infrared spectrum of 2 (R = H).

The structure of an α-hydroxy acid for 2 (R = H) consistent with the pK_a value (see Experimental), was proved by the mass spectrum of the methyl ester 2 (R = CH_3). It confirmed the molecular weight of 233, showed a strong peak at *m/e* 174 due to loss of the carbomethoxy group and an intense peak at *m/e* 144. The latter indicates fragmentation of the molecule at its labile benzylic-type carbon–carbon bond.

(4) The tautomerism of 2-amino-2-oxazolin-4-ones has been investigated recently and we therefore prefer to write expression 1 for indolmycin rather than the alternative 2-imino-4-oxazolidinone form.

(5) C. F. Howell, N. A. Quinones, and R. A. Hardy, Jr., *J. Org. Chem.*, **27**, 1686 (1962).

(6) J. B. Brown, H. B. Henbest, and E. R. H. Jones, *J. Chem. Soc.*, 3172 (1952).

α-Indolmycenic acid (**2**, R = H) on reduction with lithium aluminum hydride gave the glycol **3**. This was oxidized with sodium periodate by the method of Brown, *et al.*,[6] to the optically active aldehyde **4**.

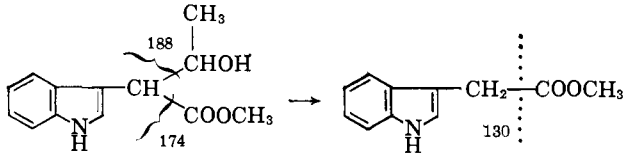
The aldehyde **4** was obtained also from β-indolmycenic acid (**5**, R = H) by the same sequence of reactions. This proved that α- and β-indolmycenic acids are epimeric at the carbon atom carrying the hydroxyl group rather than structural isomers. This is consistent with the similarity of the pK_a values.

From an incomplete hydrolysis of indolmycin (**1**) the amide **6** was isolated which on further hydrolysis yielded α-indolmycenic acid (**2**, R = H). α-Indolmycenic acid amide (**6**) was reconstituted also from **2**.

Acid hydrolysis of indolmycin (**1**) yielded, in addition to methylamine, three compounds which were stable under the reaction conditions employed. One product was soluble in 2% bicarbonate solution and the analysis showed it to be isomeric to α- and β-indolmycenic acids (**2**, **5**; R = H). This compound was obtained in 24% yield and shall be referred to as γ-indolmycenic acid. The second product was isolated in 45% yield. It was soluble in 5% sodium carbonate solution and had the composition $C_{13}H_{12}N_2O_3$. The third compound (9% yield) was not soluble in carbonate solution but had also the molecular formula $C_{13}H_{12}N_2O_3$.

Titration of the main product **7** in dioxane–water showed a $pH_{1/2}$ of 7.80. The infrared absorption spectrum in dioxane had a band at 5.47 μ and another of greater intensity at 5.67 μ in the carbonyl region. These values correspond closely to those of 2,4-oxazolidinedione (see Experimental). Alkaline hydrolysis of the compound **7** gave the amide **6** and a mixture of α- and β-indolmycenic acids (**2**, **5**; R = H).

γ-Indolmycenic acid (**8**, R = H), m.p. 157–158°, $[\alpha]^{28}D$ +63°, had to be a structural isomer of the other two acids previously isolated. Titration showed it to be a weaker acid than the α-hydroxy acids **2** and **5** (R = H). The n.m.r. spectrum of the methyl ester of **8** revealed the CH_3–C(H)(HO)–C(H)< moiety by a doublet (8.97 τ, 3 protons), a singlet (6.8 τ, 1 proton), a doublet (6.18 τ, 1 proton), and a multiplet (5.6 τ, 1 proton). The structure of γ-indolmycenic acid therefore must be **8** (R = H). This assignment was confirmed by the mass spectrum of the methyl ester **8** (R = CH_3).[7]



The carbonate-insoluble product obtained by acid degradation of indolmycin proved to be quite different from the isomeric oxazolidinedione derivative **7** and could not be an epimer. Titration under analogous conditions did not give a $pH_{1/2}$ value below 11. The infrared absorption spectrum (KBr) showed bands at 5.68 and 5.78 μ in the carbonyl region and also bands at 3.1, 8.15, 8.28, and 9.24 μ. This spectrum is consistent with an 1,3-oxazine-2,4-dione derivative.[8] Therefore structure **9** is assigned to this degradation product. This assignment was confirmed by further reactions. Compound **9** on treatment with refluxing 2 *N* sodium hydroxide solution gave β-indolylacetic acid (**10**) by retro-aldol cleavage and hydrolysis. Hydrolysis of **9** in cold 2 *N* sodium hydroxide solution or heating **9** to its point of decomposition resulted in the formation of an optically inactive compound, the amide **11**. This, on alkaline hydrolysis at elevated temperature, yielded also β-indolylacetic acid. The ultraviolet spectrum above 220 mμ of the crotonamide **11** only shows absorption characteristic of β-indolyl compounds in spite of the presence of a conjugated double bond. This seems to indicate that methyl and indolyl groups are *cis* to each other and that coplanarity of the double bond with the indole moiety is inhibited. Additional evidence for this stereochemical assignment was obtained from the n.m.r. spectrum.[9] The allylic C-methyl group in **11** (8.96 τ, doublet) appeared to be strongly shielded by the indole ring. The geometry of **11** appears to be consistent also with the proposed mechanism of degradation (Fig. 1).

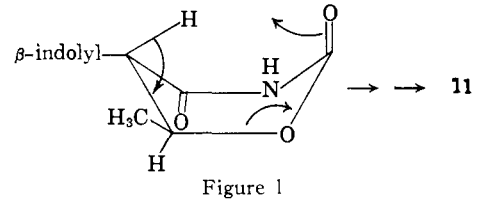
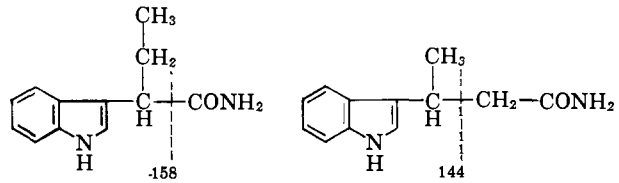


Figure 1

Hydrogenation of the double bond in **11** on a very small scale yielded a crude product, which showed a mass spectrum consistent with the structure of an α-substituted butyramide because of an intense peak at *m/e* 158.[10]



Indolmycin epimerizes to isoindolmycin (**12**), m.p. 242–245°, in alkaline solution. It also yields α- and β-indolmycenic acids (**2**, **5**; R = H) on alkaline hydrolysis. This shows that the optically active center carrying the C-methyl group is identical in both epimers. Acid degradation of isoindolmycin (**12**) gave the oxazolidinedione derivative **13** in 63% yield and a small quantity (7%) of an acid, m.p. 137°. This acid was not identified but was not identical with either α, β-, or γ-indolmycenic acid or β-indolylacetic acid. A third product (18%), insoluble in sodium carbonate solution, did not correspond to the oxazine derivative **9** but showed an infrared absorption spectrum which in the carbonyl region closely resembled that of the 2,4-oxazolidinedione **13**. While the latter, however, displayed two sharp bands at 2.90 and 2.98 μ, the carbonate-insoluble compound had only one sharp band at 2.95 μ, indicating the presence of only one –N–H group. The mass spectrum of this compound showed the parent peak at 258 and a very intense peak at 144. On the basis of these data, structure **14** is assigned to this compound.

The formation of the various products in the acid degradation of indolmycin (**1**) and its isomer **12** can be explained by postulating protonation of the exocyclic nitrogen atom as the first step (A). Hydrolysis of the methylimino group gives the oxazolidinedione derivatives **7** or **13**. Cleavage of the (5–1) C–O bond leads

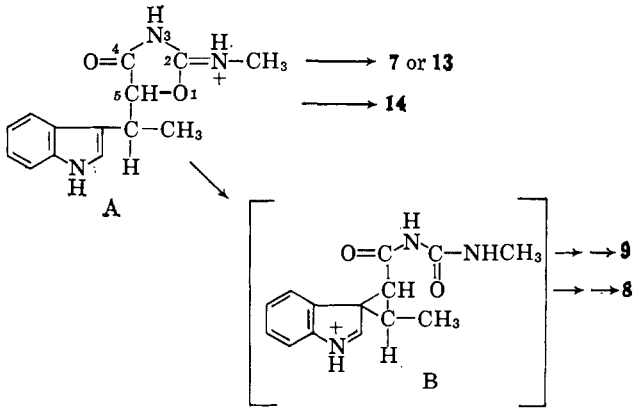
(7) In addition to the parent peak, at *m/e* 233, strong peaks were found at *m/e* 189, 188, 174, and 130. Breakage of either benzylic-type carbon-carbon bond results in fragments of mass 174 or 188, respectively. The peak at 189 indicates retro-aldol cleavage to methyl β-indolylacetate and loss of the carbomethoxyl group from this molecule gives a fragment with mass 130.

(8) E. Testa, L. Fontanella, G. Christiani, and G. Gallo, *J. Org. Chem.*, **24**, 1928 (1959).

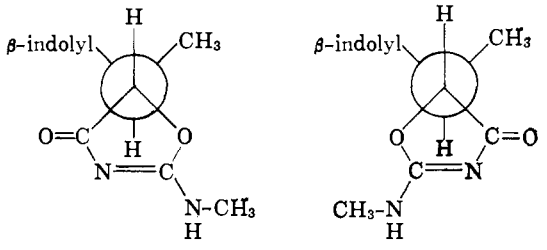
(9) We wish to thank Dr. LeRoy F. Johnson, Varian Associates, for this spectrum.

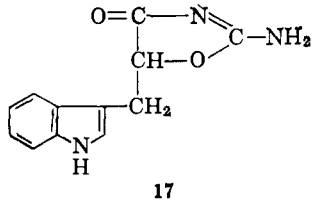
(10) For a β-substituted amide, an intense peak at 144 would have been expected from fragmentation at the benzylic-type bond.

to the intermediate B. This by internal ring closure can either revert to A or give a six-membered ring compound which loses methylamine to yield **9**. Competing with these reactions is the attack of water at the cyclopropane ring of B to yield ultimately the β-hydroxy acid **8**. Rupture of the (4–3) C–N bond, rotation around the (1–2) O–C bond, reclosure of the ring, and hydrolysis of the imino group results in compound **14**.



The different behavior of indolmycin (**1**) and its isomer **12** to acid treatment suggests that the β-indolyl group is *trans* to the (5–1) C–O bond in indolmycin (**1**) in the least hindered conformation, corresponding to **15**. Isoindolmycin (**12**), which does not rearrange with migration of the indole moiety to any appreciable extent, should then be represented by **16**.





The migration of the indole moiety under the conditions of acid degradation in indolmycin (**1**) appears to be facilitated by the presence of the C-methyl group. N-Demethyl-C-demethylindolmycin (**17**) was synthesized and subsequently degraded under analogous conditions. The expected oxazolidine-2,4-dione corresponding to **7** was isolated in high yield.

Synthetic Studies.—α-Indolmycenic acid ethyl ester (**2**, R = C_2H_5) can be prepared by stereospecific reaction from indole and ethyl *trans*-2,3-epoxybutyrate. In order to find a suitable procedure for this step, the components were allowed to react under a variety of conditions and the resulting mixture after alkaline hydrolysis was then qualitatively analyzed for α-, β-, γ-indolmycenic and β-indolylacetic acids by paper chromatographic techniques. By this method we were able to show that the two compounds when heated together in a sealed glass tube to about 260° yielded a mixture containing both α- and γ-indolmycenic acid esters (**2** and **8**, R = C_2H_5).[11] Since this reaction is catalyzed by protons, the addition of acids, such as phenol or trifluoroacetic acid, permitted lowering of the temperature to about 140°. However, both isomeric acids still were formed. Only α-indolmycenic acid ester (**2**, R = C_2H_5) was obtained when Lewis acids such as aluminum chloride or stannic chloride were used as catalyst and the components were allowed to react at low temperatures in solution. This method appeared to be the simplest, although it was discovered also that indolylmagnesium halide interacted with ethyl epoxybutyrate to yield only the α-acid ester **2** (R = C_2H_5).

Optically active α-indolmycenic acid ester (**2**, R = CH_3) when contacted with N,N′-dimethylguanidine yielded indolmycin (**1**) and, because of the alkaline reaction conditions, isoindolmycin (**12**), also.

Experimental

The melting points were determined on a Kofler block and are not corrected unless stated otherwise. The mass spectra were determined with a CEC 21-103C mass spectrometer equipped with a heated inlet system operated at 140°, electron energy 70 e.v. The n.m.r. spectra were measured in deuteriochloroform with tetramethylsilane as internal standard using a Varian Model A-60 spectrometer. The paper chromatography systems used employed a wet Whatman No. 4 paper and the upper layer of a benzene–acetic acid–water (2:1:1) mixture as running phase.

Alkaline Hydrolysis of Indolmycin (**1**).—Indolmycin (10.1 g.) was heated under reflux in 100 ml. of 10% sodium hydroxide solution under nitrogen for 30 min. After cooling to 5°, the solution was extracted with 50 ml. of ether and twice with 50 ml. of ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue, 1.07 g., was crystallized from water (m.p. 182–185°) and after four recrystallizations from the same solvent yielded 380 mg. of α-indolmycenic acid amide (**6**), m.p. 187–188°, $[\alpha]^{25}D$ −53° (*c* 1 in methanol). Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.03; H, 6.47; N, 12.84. Found: C, 66.07; H, 6.42; N, 12.30.

The basic reaction mixture was then acidified with 2 *N* sulfuric acid and extracted three times with 80 ml. of ethyl acetate. The combined ethyl acetate phases were dried over sodium sulfate, filtered, and evaporated. The residue (7.15 g.) was crystallized from water to yield 3.80 g. of crude α-indolmycenic acid (**2**). Recrystallization from the same solvent afforded the pure product, m.p. 181–182°, $[\alpha]^{25}D$ −10° (*c* 2 in methanol). Calcd. for $C_{12}H_{13}NO_3$: C, 65.90; H, 5.98; N, 6.40; C-CH_3 (one), 6.87. Found: C, 66.09; H, 5.90; N, 6.38; C-CH_3, 4.86. Titration of the acid in 50% aqueous ethanol showed a pH ½ value of 5.01, neut. equiv. 222 (calcd. 219). α-Hydroxyisobutyric acid under identical conditions showed a pH ½ of 4.95.

The aqueous mother liquors from the crystallization of α-indolmycenic acid contained a mixture of α- and β-indolmycenic acids which was isolated by evaporation of the solvent. β-Indolmycenic acid was obtained by seven recrystallizations from chloroform. After each recrystallization the mother liquor was evaporated and the infrared absorption spectrum (KBr) of the residue was compared with that of the crystalline fraction. α-Indolmycenic acid shows strong bands at 8.95, 10.20, and 10.70 μ, but none at 9.27, 9.96, and 10.96 μ while β-indolmycenic acid shows strong bands at 9.29, 9.96, and 10.96 μ but none at 8.95, 10.20, and 10.70 μ. After six recrystallizations, the spectra of the residue from the evaporated mother liquor and of the crystalline fraction were identical, indicating the β-indolmycenic acid to be pure. A final recrystallization afforded the analytical sample, m.p. 142–143.5°, $[\alpha]^{25}D$ −36° (*c* 1.8 in methanol). Calcd. for $C_{12}H_{13}NO_3$: C, 65.90; H, 5.98; N, 6.40. Found: C, 65.75; H, 6.08; N, 6.77. Titration of the acid in 50% aqueous ethanol showed pH ½ 4.91 neut. equiv. 219 (calcd. 219).

In a different experiment indolmycin (5.90 g.) was hydrolyzed in 100 ml. of boiling sodium hydroxide under nitrogen for 45 min. The reaction mixture was cooled and extracted with 50 ml. of ethyl acetate. Evaporation of the ethyl acetate phase yielded no residue. The aqueous phase was acidified with 38% hydrochloric acid and extracted three times with 50 ml. of ethyl acetate. The combined ethyl acetate phases were dried over sodium sulfate, filtered, and evaporated to yield a mixture (5.0 g.) of α- and β-indolmycenic acids, showing an optical rotation of $[\alpha]^{25}D$ −17.8° (*c* 1 in methanol). From this it was estimated that alkaline hydrolysis of indolmycin yielded the α-acid **2** and the β-acid **5** (R = H) in the ratio 2:1.

(11) A trace of β-indolmycenic acid was also detected frequently in the chromatograms, although its ester was not before hydrolysis.

In a third experiment, 1 g. of indolmycin was heated under reflux for 3.5 hr. in 50 ml. of a 10% sodium hydroxide solution and 10 ml. of a 5% barium hydroxide solution. The basic hydrolysis products were distilled in a stream of carbon dioxide free of nitrogen and trapped in 0.5 *N* hydrochloric acid. After 3.5 hr., the precipitated barium carbonate was filtered from the hydrolysis solution and dried (198 mg.).

Evaporation of the hydrochloric acid solution yielded 380 mg. of basic hydrolysis products as hydrochlorides. Crystallization from methanol afforded ammonium chloride, identified by sublimation point, infrared absorption spectrum, and conversion to 2,4-dinitroaniline.

The methanol mother liquor, containing predominantly methylamine hydrochloride, was evaporated to dryness. A slight excess of a 10% sodium hydroxide solution was added and the volatile bases were trapped in a 2% solution of 2,4-dinitrochlorobenzene. 2,4-Dinitro-N-methylaniline, m.p. 173–175°, identified by mixture melting point with an authentic sample and by infrared absorption spectrum, was obtained.

Basic Hydrolysis of α-Indolmycenic Acid Amide (6).—α-Indolmycenic acid amide (15 mg.) was heated on a steam bath in 2 ml. of a 5% sodium hydroxide solution for 90 min. The reaction mixture was extracted with methylene chloride (3 ml.), then acidified with 2 *N* sulfuric acid, and extracted three times with 8 ml. of ethyl acetate. The combined ethyl acetate phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was crystallized from chloroform–methylene chloride to yield 6 mg. of α-indolmycenic acid (**2**) which was identified by its infrared absorption spectrum.

Preparation of α-Indolmycenic Acid Amide (6).—To a solution of α-indolmycenic acid (**2**, R = H) (265 mg.) in tetrahydrofuran (2 ml.) was added a solution of carbonyldiimidazol (200 mg.) in the same solvent (1.5 ml.). Ammonia was bubbled into the cooled solution for a few minutes. The reaction mixture was diluted with water (15 ml.) and three times extracted with ether. The combined ether extracts after suitable work-up yielded α-indolmycenic acid amide (120 mg.) identified by infrared spectrum and mixture melting point.

Pyrolysis of α-Indolmycenic Acid (2).—α-Indolmycenic acid (300 mg.) was pyrolized at 200–230° in a sublimation tube at 0.05 mm. A small amount of crystalline sublimate was obtained and purified by repeated, careful sublimation at 40–50°. The material melted at 50–52° and was identified with indole by mixture melting point, 50–52°, with an authentic sample and by its infrared spectrum.

Permanganate Oxidation of α-Indolmycenic Acid (2).—α-Indolmycenic acid (1 g.) was dissolved in 200 ml. of water at 50°; 12 ml. of 2 *N* sulfuric acid and 200 ml. of 5% potassium permanganate solution were added in 50-ml. portions over a period of 6 hr. After standing at room temperature for 20 hr., the pH was adjusted to 7.5 and the excess permanganate was destroyed by addition of sodium bisulfite. The manganese dioxide was separated by filtration through Supercel and the filtrate was concentrated to 100 ml. Extraction with five 100-ml. portions of ether yielded 20 mg. of crude anthranilic acid as a yellow oil, which crystallized from water in plates, m.p. 142–145°. This material was identified by melting point, mixture melting point, and paper chromatographic comparison with authentic anthranilic acid.

Methyl Ester of α-Indolmycenic Acid.—α-Indolmycenic acid (3.3 g.) was heated under reflux in 80 ml. of methanol and 5 ml. of concd. sulfuric acid for 17 hr. The cooled solution was poured into ice–water (500 g.) and extracted four times with ethyl acetate (100 ml. each). The combined ethyl acetate phases were washed with 50 ml. of a 5% sodium bicarbonate solution and then with 50 ml. of water, washed with sodium bicarbonate solution, and then with 50 ml. of water, dried over sodium sulfate, filtered, and evaporated to dryness. The residue was distilled at 190–205° (bath temperature) and 0.01 mm., yield 2.75 g. The mass spectrum showed that the methyl ester was contaminated with about 10% of a substance of mol. wt. 247, presumably the methyl ester methyl ether. After standing for several days, the oil crystallized. Recrystallization from ether–hexane yielded a product melting at 82–83°, which was shown by mass spectrometry to be pure α-indolmycenic acid methyl ester, $[\alpha]^{25.7}D$ +6° (*c* 1 in methanol). Calcd. for $C_{13}H_{15}NO_3$: C, 66.93; H, 6.48; N, 6.01. Found: C, 67.00; H, 6.48; N, 5.92.

Reduction of α-Indolmycenic Acid (2) to the Glycol 3.—α-Indolmycenic acid (3.8 g.) was added to a stirred mixture of 3.9 g. of lithium aluminum hydride in 100 ml. of ether. Water (20 ml.) was slowly added after 30 min., and the mixture acidified with 2 *N* sulfuric acid. After separation of the two phases the aqueous layer was extracted three times with 25 ml. of ether and the combined ether fractions first with 25 ml. of a 5% bicarbonate solution, then with 25 ml. of water. The ether solution was dried over sodium sulfate, filtered, and evaporated to dryness. The residue distilled at 200–240° (bath temperature) and 0.08 mm. to yield the glycol **3** as a yellow oil (2.36 g.) which crystallized on prolonged standing; m.p. 88–91°, $[\alpha]^{25}D$ −28.3° (*c* 2 in methanol). Calcd. for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.44; H, 7.37; N, 7.20.

α-(β-Indolyl)-propionaldehyde (5).—A solution of 505 mg. of the glycol **3** in 8 ml. of methanol was added to a stirred mixture of 0.1 *N* aqueous sodium periodate solution (25 ml.), light petroleum ether (15 ml., b.p. 40–60°), and ether (15 ml.) at 14° under nitrogen. After 12 min., the phases were separated and the aqueous layer was extracted twice with 10 ml. of ether. The combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue which showed an infrared absorption spectrum ($CHCl_3$) corresponding to an aldehyde (bands at 3.6, 3.7, 5.83 μ), was dissolved in 5 ml. of methanol and added to a solution of semicarbazide hydrochloride (1.31 g.) and fused sodium acetate (922 mg.) in water (10 ml.). After standing for 2 hr. at room temperature, the solution was concentrated *in vacuo* to 10 ml. and extracted three times with 10 ml. of ether. The combined ether phases were extracted with 10 ml. of water, then with 10 ml. of a 5% sodium bicarbonate solution, and again with 10 ml. of water, dried over sodium sulfate, filtered, and evaporated to dryness. The crude semicarbazone was crystallized from ethanol and after a few recrystallizations from the same solvent yielded the pure compound; 115 mg., m.p. 182°, $[\alpha]^{25}D$ −23 ± 2° (*c* 1 in methanol). Calcd. for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33; C-CH_3 (one), 6.54. Found: C, 62.78; H, 6.25; N, 23.91; C-CH_3, 5.99.

α-(β-Indolyl)-propionaldehyde (4) from β-Indolmycenic Acid.—β-Indolmycenic acid (**5**, R = H) (480 mg.) was added to a stirred mixture of 1.2 g. of lithium aluminum hydride in 100 ml. of ether under nitrogen. After 30 min. water (25 ml.) was added, the mixture acidified with concentrated hydrochloric acid, and the phases were separated. The aqueous layer was extracted twice with 20 ml. of ether and the combined ether phases were washed with 25 ml. of a 10% sodium bicarbonate solution, dried over sodium sulfate, filtered, and evaporated to dryness. The residue was distilled in a sublimation apparatus at 200–220° (bath temperature) and 0.2 mm. to yield the glycol epimeric to **3** as a yellow oil which was used without further purification.

The crude glycol was dissolved in 10 ml. of methanol and added to a stirred mixture of 0.1 *N* sodium periodate solution (2.5 ml.), ether (15 ml.), and petroleum ether (15 ml.) (b.p. 40–60°) under nitrogen. After 10 min. the upper phase was removed and the lower phase washed twice with 10 ml. of ether. The combined organic layers were dried over sodium sulfate, filtered, and evaporated to dryness to yield the aldehyde **4** as an oil (250 mg.) showing an identical infrared absorption spectrum to that of the aldehyde obtained from degradation of α-indolmycenic acid. The residue was dissolved in 5 ml. of methanol and added to a solution of semicarbazide hydrochloride (1.3 g.) and fused sodium acetate (1 g.) in water (10 ml.). After standing at room temperature for 17 hr., the solution was concentrated *in vacuo* to 8 ml., water (10 ml.) was added, and the solution extracted three times with 10 ml. of ether. The combined ether phases were washed with 10 ml. of water then with 10 ml. of a 5% sodium bicarbonate solution and again with 10 ml. of water, dried over sodium sulfate, filtered, and evaporated to dryness. The crude semicarbazone was crystallized from ethanol (70 mg.). Recrystallization from the same solvent yielded a product melting at 182–184°, which was shown to be identical with the semicarbazone obtained from degradation of α-indolmycenic acid by mixture melting point and infrared absorption spectrum; $[\alpha]^{25}D$ −26 ± 2° (*c* 1 in methanol).

Acid Degradation of Indolmycin (1).—Indolmycin (20 g.) was added to refluxing 2 *N* sulfuric acid (900 ml.) under a nitrogen atmosphere. After 30 min., the solution was cooled in an ice bath and then extracted three times with 200 ml. of ethyl acetate. The organic phases were combined and extracted three times with 200 ml. of a 2% bicarbonate solution. The extraction of the organic phase was repeated three times with 5% sodium carbonate solution (200 ml.). The combined ethyl acetate phases were filtered after drying over sodium sulfate and evaporated to dryness. The residue was crystallized from ethanol to yield 5-(β-indolyl)-6-methyl-1,3-oxazine-2,4-dione (**9**) as colorless crystals, m.p. 178–188° dec., Kofler block; 1.751 g., 9% yield. The product after recrystallization from acetone–ethanol had a melting point of 230–232° dec. The observation was made that crystals, which melted on the Kofler block at 180–205°, melted in a capillary tube at 232–235° dec., $[\alpha]^{24}D$ −9.9° (*c* 1 in dioxane). Calcd. for $C_{13}H_{12}N_2O_3$: C, 63.92; H, 4.95; N, 11.47; C-CH_3 (one), 6.16. Found: C, 63.74; H, 4.97; N, 11.54; C-CH_3, 6.11.

The combined sodium carbonate solutions were acidified to pH 2 with 2 *N* sulfuric acid and extracted three times with 160 ml. of ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was crystallized from chloroform to yield 5-α-(β-indolyl)-ethyl-oxazolidinedione-2,4 (**7**); 8.6 g. (45%), m.p. 171–177°; after recrystallization m.p. 178–179.5°; λ_{max} (dioxane) 5.47, 5.67 μ. Calcd. for $C_{13}H_{12}N_2O_3$: C, 63.92; H, 4.95; N, 11.47. Found: C, 63.29; H, 4.89; N, 11.64. Titration in aqueous dioxane (25.6 mg. in 20 ml. of dioxane and 10 ml. of water)

showed pH $^1/_2$ 7.80, neut. equiv. 239 (calcd. 244). 2,4-Oxazolidinedione (λ_{max}, dioxane, 5.42, 5.65 μ) under the same conditions gave a pH $^1/_2$ of 7.92.

The combined sodium bicarbonate solutions were acidified with 2 *N* sulfuric acid and extracted three times with 120 ml. of ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was crystallized from chloroform to yield α-(β-indolyl)-β-hydroxybutyric acid (**8**, R = H), m.p. 146–153°, 3.95 g. (24%). Recrystallization from the same solvent furnished an analytical sample, m.p. 157–158°. Calcd. for $C_{12}H_{13}NO_3$: C, 65.9; H, 5.98; N, 6.40. Found: C, 66.02; H, 6.12; N, 6.21. Titration in aqueous ethanol (50%) showed pH $^1/_2$ 6.0, neut. equiv. 220 (calcd. 219), $[\alpha]^{25.5}D$ +63° (*c* 1 in methanol).

Hydrolysis of 5-α-(β-Indolyl)-ethyloxazolidinedione-2,4 (7).—The oxazolidinedione derivative **7** (2 g.) was added to 50 ml. of a 0.5 *N* sodium hydroxide solution and stirred at 75° under nitrogen for 18 hr. After cooling, the solution was extracted twice with 50 ml. of ethyl acetate. The organic phase was dried over sodium sulfate, filtered, and evaporated to dryness. The residue (367 mg.) was crystallized from water to yield 161 mg. of α-indolmycenic acid amide (**6**), m.p. 180–186°, identified by infrared absorption spectrum.

The basic aqueous phase was acidified with 2 *N* sulfuric acid and extracted three times with 50 ml. of ethyl acetate. The combined ethyl acetate phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was crystallized from water to yield 711 mg. of α-indolmycenic acid (**2**, R = H), m.p. 180°. The mother liquor was shown to contain both α- and β-indolmycenic acids (**2**, **5**, R = H) by paper chromatographic comparison with authentic samples.

Methyl Ester of γ-Indolmycenic Acid (8, R = CH_3).—γ-Indolmycenic acid (**8**, R = H; 926 mg.) was dissolved in 50 ml. of ether and treated with an excess of a solution of diazomethane in ether. After 5 min., 1 ml. of acetic acid was added and the solution extracted with 25 ml. of a 5% sodium bicarbonate solution and twice with 25 ml. of water. After drying over sodium sulfate and filtration, the organic phase was evaporated to dryness. The residue was distilled at 200° (bath temperature) and 0.01 mm. A yellow glass (700 mg.) was obtained which crystallized on standing. Recrystallization from ether–hexane yielded the pure methyl ester, m.p. 85–87°. Calcd. for $C_{13}H_{15}NO_3$: C, 66.93; H, 6.48; N, 6.01; OCH_3, 13.30. Found: C, 66.58; H, 6.45; N, 7.50; OCH_3, 13.50.

3-Indolylacetic Acid from 5-(β-Indolyl)-6-methyl-1,3-oxazine-2,4-dione (9).—The oxazine derivative **9** (60 mg.) was heated under reflux in 3 ml. of a 10% sodium hydroxide solution under nitrogen for 2 hr. The cooled solution was acidified with 2 *N* sulfuric acid and extracted twice with 5 ml. of ethyl acetate. The combined ethyl acetate phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue crystallized from chloroform to yield indolylacetic acid (2.6 mg.), identified by its infrared absorption spectrum.

α-(β-Indolyl)-crotonamide (11).—(a) The oxazine derivative **9** (102 mg.) was dissolved at room temperature in 10 ml. of a 0.5 *N* sodium hydroxide solution. Crystals precipitated soon. These were filtered after 18 hr., washed with water and dried *in vacuo*; 59 mg., 70%, m.p. 154–155°. Recrystallization from ethyl acetate–ether raised the melting point to 156–157°, $[\alpha]^{25}D$ 0° (*c* 2 in methanol). Calcd. for $C_{12}H_{12}N_2O$: C, 71.98; H, 6.04; N, 13.99; C-CH_3 (one), 7.50; mol. wt., 200. Found: C, 71.83; H, 5.94; N, 13.89; C-CH_3, 6.48; mol. wt. (determined by mass-spectrometry), 200.

(b) The oxazine derivative **9** (212 mg.) was heated to its decomposition point under nitrogen and held at this temperature (235–245°) for 10 min. After cooling, the crude decarboxylated product was crystallized from 2.5 ml. of ethyl acetate; 81 mg., m.p. 150–153°. From the mother liquor a second fraction was crystallized; 55 mg., m.p. 137–149°.

β-Indolylacetic Acid from α-(β-Indolyl)-crotonamide.—α-(β-Indolyl)-crotonamide 11 (55 mg.) was heated under reflux in 5 ml. of a 2 *N* sodium hydroxide solution for 2 hr. The sample was completely dissolved after 1 hr. After standing for 65 hr., the solution was diluted to 60 ml. with water and twice extracted with 25 ml. of ethyl acetate. The aqueous phase was acidified with 2 *N* sulfuric acid and extracted twice with 25 ml. of ethyl acetate. The last two organic phases were combined, dried over sodium sulfate, filtered, and evaporated to dryness. The residue was dissolved partly in hot benzene, filtered and the filtrate concentrated to dryness. The residue was sublimed twice at 140° and 0.02 mm. and recrystallized twice from benzene to yield indolylacetic acid (8 mg.), m.p. 162–167°, identified by its infrared absorption spectrum.

Hydrogenation of α-(β-Indolyl)-crotonamide.—The crotonamide derivative **11** (4.6 mg.) was hydrogenated in 10 ml. of methanol over 25 mg. of palladium for 15 min. at room temperature and normal pressure. The reaction mixture was filtered and the solution evaporated. The residual oil (3.5 mg.) was dissolved in chloroform–methanol, some carbon black was added, and the solution filtered and evaporated to dryness. The residue (1.6 mg.) was used for mass spectrometry. The spectrum showed the parent peak at *m/e* 202 and a very intense peak at *m/e* 158.

Isoindolmycin (12).—Indolmycin (**1**) was heated under reflux in 50 ml. of a 9% sodium ethylate solution for 1 hr. After cooling, 200 ml. of ethyl acetate was added and the solution extracted three times with 50 ml. of water. The organic phase was dried over sodium sulfate, filtered, and evaporated to dryness. The residue (2.73 g.) contained 39% indolmycin judged by its biopotency. Crystallization from methanol yielded isoindolmycin (0.53 g., m.p. 235–238°) and recrystallization from the same solvent an analytical sample, m.p. 242–245°, $[\alpha]^{25}D$ 47° (*c* 1 in methanol). Calcd. for $C_{14}H_{15}N_3O_2$: C, 65.35; H, 5.87; N, 16.33. Found: C, 65.14; H, 5.88; N, 16.67. The sample showed no bioactivity. This compound may be prepared also by treatment of indolmycin with hot sodium carbonate solution.

Acid Degradation of Isoindolmycin (12).—Isoindolmycin (**12**) (1.03 g.) was heated in 50 ml. of refluxing 2 *N* sulfuric acid under nitrogen for 25 min. After cooling, the solution was extracted twice with 50 ml. of ethyl acetate. The combined organic phases first were extracted twice with 25 ml. of a 2% bicarbonate solution and then three times with 25 ml. of a 5% sodium bicarbonate solution.

The ethyl acetate phase was dried over sodium sulfate, filtered, and evaporated to dryness. The residue was dissolved in 25 ml. of ether, filtered, and concentrated to 5 ml. Isoindolmycin (**12**) crystallized (13 mg.) and was separated and identified by its infrared absorption spectrum. The mother liquor was evaporated to dryness and the residue (220 mg.) crystallized from carbon tetrachloride–methanol to yield 50 mg. of the oxazolidinedione derivative **14**, m.p. 168–172°. An analytical sample, m.p. 169–172°, was obtained by sublimation at 120° and 0.2 mm. Calcd. for $C_{14}H_{14}N_2O_3$: C, 65.10; H, 5.46; N, 10.85. Found: C, 64.97; H, 5.46; N, 10.98. The mass spectrum showed the parent peak at *m/e* 258 and a very strong peak at *m/e* 144.

The combined carbonate solutions were acidified with 2 *N* sulfuric acid and extracted three times with ethyl acetate (50 ml.). The combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue crystallized from chloroform (620 mg., m.p. 132–136°). Recrystallization from the same solvent and subsequent drying at 80°, 2 mm., 18 hr., yielded a sample, m.p. 135–142°, which appeared to contain $^1/_6$ mole of chloroform. Recrystallization from benzene afforded samples (m.p. 135–142°) which also appeared to crystallize with $^1/_6$ mole of solvent. The mass spectrum showed mol. wt. 244 (mol. wt. for $C_{13}H_{12}N_2O_3$ 244). Calcd. for $C_{13}H_{12}N_2O_3 \cdot {}^1/_6C_6H_6$: C, 65.36; H, 5.09; N, 10.90. Found: C, 65.17; 65.03; 65.13; H, 5.57; 5.39; 5.23; N, 11.27. Calcd. for $C_{13}H_{12}N_2O_3 \cdot {}^1/_6CHCl_3$: C, 59.87; H, 4.64; N, 10.61. Found: C, 59.77; 59.82; H, 4.76; 4.62; N, 10.96.

The combined bicarbonate solutions were acidified with 2 *N* sulfuric acid and extracted three times with 20 ml. of ethyl acetate. The combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue, a reddish oil (70 mg.), yielded a small amount of crystalline material, 6 mg., after treatment with chloroform; m.p. 137°. The infrared absorption spectrum of this compound was not identical with those of the three known indolmycenic acids.

The original acidic reaction mixture, after it had been extracted with ethyl acetate, was made strongly alkaline by addition of 60 g. of potassium hydroxide, while a stream of nitrogen was bubbled first through this mixture and then through 100 ml. of a 1% alcoholic solution of 2,4-dinitrofluorobenzene. After 16 hr., the alcoholic solution was evaporated and the residue treated with ethyl alcohol to yield crystals (480 mg.), m.p. 169–175°, which were identified as N-methyl-2,4-dinitroaniline by mixture melting point with an authentic sample and its infrared absorption spectrum.

Alkaline Hydrolysis of 13.—The oxazolidinedione derivative **13** (40 mg.) was heated under reflux in 6 ml. of a 2 *N* sodium hydroxide solution under a stream of nitrogen. The nitrogen, after passing the reaction flask, was bubbled through a trap containing a 1% alcoholic solution of 2,4-dinitrofluorobenzene. Although the reaction mixture was only refluxed for 1.5 hr., the flow of nitrogen was maintained for another 16 hr. at room temperature. The aqueous reaction mixture was then acidified with 2 *N* sulfuric acid and extracted four times with 6 ml. of chloroform. The combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue yielded crystals from water (11 mg., m.p. 170–175°; $[\alpha]^{25}D$ −10.8 ± 2°, *c* 0.8 in methanol) which showed an infrared absorption spectrum identical with that of α-indolmycenic acid (**2**). Recrystallization from water furnished crystals, m.p. 181°, which showed no melting point depression on admixture to α-indolmycenic acid (**2**). The combined mother liquors contained both α- and β-indolmycenic acids as shown by paper chromatographic comparison with authentic samples.

From the 2,4-dinitrofluorobenzene solution, 2.5 mg. of crystals (m.p. 181°) were isolated which were identified as 2,4-dinitroaniline by mixture melting point with an authentic sample and by its infrared absorption spectrum.

C-Demethyl-N-demethylindolmycin (17) was prepared from guanidine and methyl β-indolylacetate in analogy to similar compounds[12]; crystals from ethanol, m.p. 239–241°. Calcd. for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; N, 18.33. Found: C, 63.09; H, 4.58; N, 18.36.

Acid Degradation of C-Demethyl-N-demethylindolmycin (17).—Compound **17** (253 mg.) was treated as described for isoindolmycin. The ethyl acetate phase after extraction with carbonate and bicarbonate solution yielded an oil (9 mg.) which showed an infrared absorption band ($CHCl_3$) at 3.45 and 5.76 μ. The 2% sodium bicarbonate fraction contained an oil (28 mg.) that showed infrared absorption at 5.5 (m) and 5.7 (s) μ (dioxane) and was judged to be an impure oxazolidine-2,4-dione. The carbonate solution yielded crystals (218 mg.), m.p. 152–159°; λ_{max} 5.52, 5.75 μ. An analytical sample was obtained from chloroform, m.p. 158–160°. Calcd. for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17. Found: C, 62.22; H, 4.32; N, 12.03.

Preparation of Racemic α-Indolmycenic Acid (2, R = H).—A mixture of ethyl 2,3-epoxybutyrate[13] (16.1 g.) and indole (16 g.) in carbon tetrachloride (50 ml.) was cooled to −10°. A solution of stannic chloride (33 g.) in carbon tetrachloride (100 ml.) was added over a period of 2 hr. After 1 hr. a reddish gum formed and more solvent (150 ml.) was added. The reaction mixture was stirred with cooling for another hour. Concentrated sodium bicarbonate solution was added until the aqueous layer remained basic. The mixture was filtered and the phases were separated. The organic solution was concentrated to yield an oil (24 g.). This was distilled at 0.3 mm. The fractions boiling below 145° were discarded and those boiling between 145 and 190° (mostly 175°) were collected; 5.45 g. The vapor phase chromatogram indicated the mixture to contain about 9% indole and 80% α-indolmycenic acid ester. The latter v.p.c. fraction was collected and analyzed. The infrared spectrum ($CHCl_3$) was identical with that of α-indolmycenic acid ethyl ester (**2**, R = C_2H_5), prepared from the optically active acid **2** and ethanol in the presence of sulfuric acid. Calcd. for $C_{14}H_{17}NO_3$: C, 67.99; H, 6.93; N, 5.66. Found: C, 68.30; H, 7.05; N, 5.62.

A sample (550 mg.) of the racemic α-indolmycenic acid ester was hydrolyzed in routine fashion and racemic α-indolmycenic acid (**2**, R = H) was obtained; 160 mg., m.p. (from H_2O) 170°. The infrared spectrum (KBr) was very similar to that of the optically active acid **2** (R = H) with slight differences in the 13 μ region. Calcd. for $C_{12}H_{13}NO_3$: C, 65.90; H, 5.98; N, 6.40. Found: C, 65.76; H, 6.00; N, 6.35.

(12) C. F. Howell, N. Q. Quinones, and R. A. Hardy, Jr., *J. Org. Chem.*, **27**, 1679 (1962).

(13) W. D. Emmons and A. J. Pagano, *J. Am. Chem. Soc.*, **77**, 89 (1955).

Preparation of Indolmycin (1) and Isoindolmycin (12).—N,N'-Dimethylguanidine hydriodide (1 g.) was dissolved in 6 ml. of a 0.7 *N* methanolic sodium methylate solution and added to 1 g. of optically active α-indolmycenic acid methyl ester. After standing at room temperature for 1 day, the solution was concentrated to 1 ml. *in vacuo*, 1 *N* hydrochloric acid (10 ml.) was added, and the mixture was extracted three times with 8 ml. of ethyl acetate. The combined organic phases were extracted twice with 10 ml. of water, once with 10 ml. of a 5% sodium bicarbonate solution, and again with 10 ml. of water, then dried over sodium sulfate, filtered, and evaporated *in vacuo* to dryness to yield an oily product (860 mg.). From this on attempted crystallization from ethyl acetate, 20 mg. of crude isoindolmycin (**12**), m.p. 224–230°, identified by infrared absorption spectrum, was isolated. The mother liquor of these crystals was evaporated *in vacuo* to dryness and the residue chromatographed on a column of Woelm aluminum oxide, nonalkaline, grade 1, starting with chloroform as eluent but after 100 ml. had been used, changing gradually to first ethyl acetate and then from ethyl acetate to ethanol, taking fractions of 18 ml. Each second fraction was investigated for its contents of indolmycin by paper chromatography. Indolmycin was eluted by ethyl acetate–5% ethanol and solvent mixtures of higher ethanol content. All fractions showed more than one component. The fractions eluted with ethyl acetate–15% ethanol contained the highest proportion of indolmycin and were investigated further. Direct crystallization from ethyl acetate yielded isoindolmycin, identified by melting point (230–235°) and infrared absorption spectrum. The mother liquor was streaked on untreated Whatman No. 4 paper and chromatographed using 2% aqueous dipotassium phosphate solution as mobile phase. After the chromatogram had been developed, the streak corresponding in R_f value to indolmycin was cut out and eluted with methanol. The methanolic solution was concentrated *in vacuo* to 10 ml., diluted with 100 ml. of ethyl acetate, and extracted twice with 20 ml. of water. The organic phase was dried over sodium sulfate, filtered, and evaporated to dryness. The residue (1.5 mg.) crystallized from ethyl acetate and was washed with ethyl acetate and ether; m.p. 206–209°, on admixture to indolmycin (m.p. 210–213°), m.p. 207–212°. The infrared absorption spectrum (KBr) was identical with that of an authentic sample. The potassium bromide pellet was dissolved in 10 ml. of water. The solution was analyzed by ultraviolet absorption spectrum for indolmycin and by biological assay. Both methods gave identical results of 0.05 mg. of indolmycin per ml.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN 1, N. Y.]

Optically Active Polyamides. Poly-D(−)-β-methyl-ε-caprolactam[1]

BY C. G. OVERBERGER AND H. JABLONER[2]

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D(−)-β-Methyl-ε-caprolactam has been polymerized to a crystalline polymer melting 90° above the polymer prepared from the racemic monomonomer. A study of the solution properties of poly-D(−)-β-methyl-ε-caprolactam and a model compound, (+)-6-acetamido-3,N-dimethylhexanamide, in mixtures of cresol and chloroform have shown that no ordered helix structure exists, that solvation of the amide carbonyl is independent of polymer conformation, and that each mer is solvated independently. In addition, theories have been presented to explain the changes in λ_e and $[\eta]$ with solvent composition.

While optically active polymers of α-amino acids have been extensively investigated, optically active polymers other than the poly-α-amino acids have, until recently, received only a small amount of attention.

There have been two principal approaches in the study of optically active polymers. One approach has been concerned primarily with a symmetric induction during polymerization; the second had been concerned with the properties of optically active polymers prepared from optically active monomers. It is only with the second approach that we are concerned here.

In general, optically active polymers have higher melting points and higher crystallinity than their racemic analogs. Examples of this effect are polypropyleneimine,[3] propylene oxide,[4] poly-2-methylbutanal,[5] poly-3-methyl-1-pentene,[6] and polyesters of decamethylene glycol and *d*- and *meso*-tartaric acids.[7] Optically

(1) A preliminary communication on this subject was published: *J. Polymer Sci.*, **55**, 532 (1961).

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