The Absolute Configuration of Indolmycin

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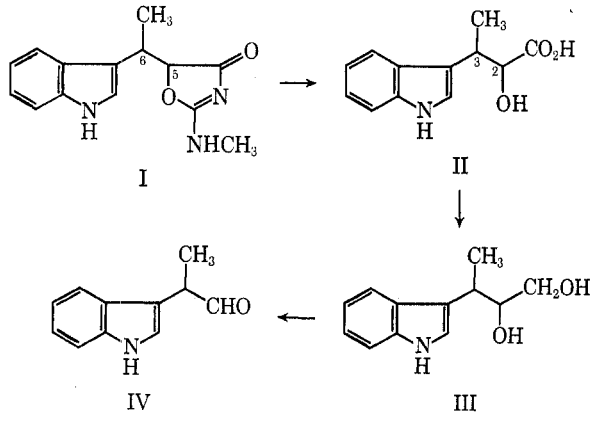
Synthesis of the enantiomer of α-indolmycenic acid from (−)-*trans*-2,3-epoxybutyric acid establishes the absolute configuration of the antibiotic indolmycin as 5*S*,6*R*. The absolute configuration of the plant growth hormone (*S*)-(+)-α-(3-indolyl)propionic acid, assigned earlier by the quasiracemate method, has been confirmed by direct correlation with α-indolmycenic acid.

Indolmycin (**1**), an antibiotic isolated[1] from an African strain of *Streptomyces albus*, is of interest for its antimicrobial activity[2] against strains of staphylococci which are resistant to many commercially available antibiotics. The structure was elucidated in a careful study by Schach von Wittenau and Els,[3] who also were able to deduce the relative configuration and to accomplish a total synthesis of the antibiotic. The ordinarily intimate relationship of biological activity to configuration of chiral molecules prompted this study of the absolute configuration of indolmycin.

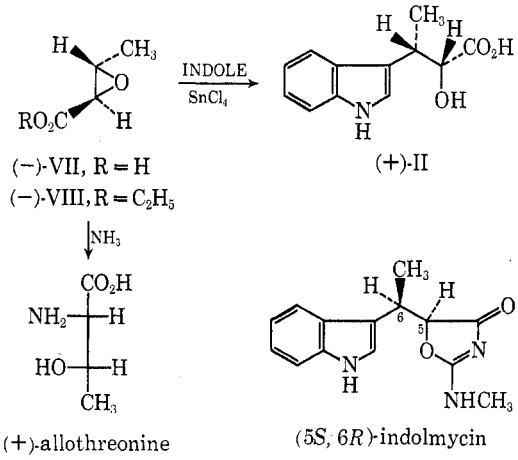
Alkaline hydrolysis of indolmycin was reported[3] to yield a mixture of (−)-α-indolmycenic acid (II) and its epimer at the carbinol carbon C-2, β-indolmycenic acid. Reduction with lithium aluminum hydride afforded glycol III, which was cleaved with periodate to the optically active aldehyde IV. The relative configuration at the two asymmetric centers was deduced from the behavior of indolmycin and its 5 epimer on acid hydrolysis, and confirmed by synthesis of racemic II from indole and *trans*-ethyl-2,3-epoxybutyrate. Consequently, any of the degradation products II–IV could be used for determination of absolute configuration by correlation with a compound of known configuration.



The paucity of optically active compounds of rigorously established configuration containing a 3-indole substituent attached to the asymmetric center limited efforts to use aldehyde IV in a correlation scheme; preliminary attempts to prepare (+)- or (−)-IV by reduction of derivatives of α-(3-indolyl)propionic acid (VI) or by oxidation of 2-(3-indolyl)propan-1-ol (V) were frustrated by low yields and racemization. We turned therefore to direct synthesis of indolmycenic acid (II) from a compound of known configuration, and chose (−)-*trans*-2,3-epoxybutyric acid (VII) for this purpose.

(−)VII, $[\alpha]^{25}D$ −74.8°, was prepared by resolution of the racemic acid with brucine according to the procedure of Harada and Oh-hashi.[4] These workers established the absolute configuration of (−)-VII as (2*R*,3*S*) by nucleophilic opening of the epoxide ring with ammonia to give (+)-(2*S*,3*S*)-allothreonine.[5] Levorotatory VII was converted to the ethyl ester (VIII) by reaction of the silver salt with ethyl iodide. The stannic chloride catalyzed reaction between (±)-VIII and indole was reported by Schach von Wittenau and Els[3] to yield only (±)-α-indolmycenic acid (II) and none of the diastereoisomer; it may be pictured as nucleophilic displacement with inversion at C-3 of the coordinated epoxide. Regardless of detailed mechanism, the configuration at C-2 in α-indolmycenic acid must be the same as that in VIII.

Indole reacted with (−)-VIII in carbon tetrachloride at −10°, with stannic chloride as catalyst, to give α-indolmycenic acid ethyl ester, which was hydrolyzed to the crystalline optically active acid II, $[\alpha]D$ +7.8°. The synthetic acid gave infrared and nmr spectra identical with those of the hydrolysis product of indolmycin, but the rotation was the opposite of that reported. Since dextrorotatory II prepared by synthesis from (−)-VII must have the (2*R*,3*S*) configuration, (−)-II is the (2*S*,3*R*) isomer and indolmycin correspondingly has the (5*S*,6*R*) configuration.



α-(3-Indolyl)propionic Acid.—The establishment of absolute configuration of indolmycin also permitted an unambiguous chemical corroboration of the absolute configuration of α-(3-indolyl)propionic acid ("indole-isopropionic acid") (VI), a plant growth hormone.

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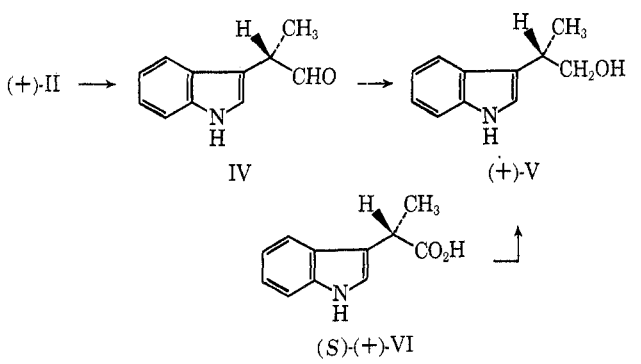
Acid VI is the higher homolog of a long-known plant growth regulator, indoleacetic acid (heteroauxin) and has been shown in a number of studies[6,7] to have auxin activity often equal to or surpassing that of indoleacetic acid. The dextrorotatory isomer has been isolated[8] both in the free state and as its mannitol ester from the sclerotia and saprophytic cultures of the ergot fungus *Claviceps purpurea*. The biosynthesis of VI from tryptophan and methionine has been the subject of a recent investigation.[9]

The enantiomers of VI show clear differences in hormonal activity, the (+) isomer being more active in most tests;[7] this finding has been attributed to differences in diffusion velocity in the plant.[7a] The effect of configuration on auxin activity may be important for the understanding of the growth-regulating mechanism, and the configuration of optically active VI has been the subject of earlier investigations. Sjöberg[10] used the quasiracemate method to deduce the (*S*) configuration for (+)-VI, employing α-(1-naphthyl) propionic acid as the reference standard, and later[11] came to the same conclusion on the basis of comparison of ORD curves of VI and related acids.

To permit a rigorous assignment by chemical interconversions, α-(3-indolyl) propionic acid (VI) was prepared and resolved with brucine. Reduction of (+)-VI, $[\alpha]_D$ +106°, with diborane gave (+)-2-(3-indolyl)propan-1-ol (V), $[\alpha]_D$ +28°. This alcohol was then prepared independently from (+)-α-indolmycenic acid (II). Following the degradation scheme of Schach von Wittenau and Els,[3] (+)-II was reduced to glycol III with lithium aluminum hydride. Periodate cleavage of III afforded α-(3-indolyl)propionaldehyde (IV) which was immediately reduced with lithium aluminum hydride. The reduction product,



(+)-V, was identical with the alcohol derived from (+)-VI. This correlation of (+)-VI with (+)-(2*R*,-3*S*)-II confirms the (*S*) configuration assigned earlier to (+)-VI.

Experimental Section

trans-**2,3-Epoxybutyric Acid (VII).**—Acid VII was prepared by epoxidation of sodium crotonate with hydrogen peroxide and sodium tungstate, according to the procedure of Payne and Williams.[12] The acid had mp 82–85° (lit.[12] mp 83°). Partial resolution with brucine was achieved by the published procedure.[4] The levorotatory acid had mp 53–56°, $[\alpha]^{25}_D$ −74.8° (*c* = 1, benzene); lit.[4] mp 61°, $[\alpha]^{25}_D$ −82.5° (*c* 0.59, benzene).

(−)-**Ethyl** *trans*-**2,3-Epoxybutyrate (VIII).**—A solution of 5.95 g of the brucine salt of (−)-VII, mp 175° dec, $[\alpha]^{25}_D$ −24.4° (*c* = 1.5, H_2O), in 30 ml of water was treated with 12 ml of 1 *N* sodium hydroxide solution and filtered to remove brucine. To the filtrate was added a solution of 2.04 g of silver nitrate in 6 ml of water followed by 40 ml of methanol. After cooling, the precipitated silver salt (1.23 g), mp 180–182°, was collected and dried *in vacuo*.

A mixture of the silver salt (1.68 g) and ethyl iodide (1.5 ml) in 50 ml of benzene was refluxed 40 min under nitrogen, then cooled and filtered. Distillation of the filtrate gave the ester[13] as a colorless oil: bp 110° (30 mm); 0.68 g; $[\alpha]^{25}_D$ −23.5° (*c* 2, CCl_4); ν CCl_4 1750 cm^{-1}; nmr (CCl_4) δ 1.3, t, 3 H, 1.35, d, 3 H, 3.1, m, 2 H, 5.2 q, 2 H.

(+)-α-**Indolmycenic Acid (II).**—To a solution of 0.68 g of (−)-VIII and 1.3 g indole in 10 ml of carbon tetrachloride, cooled to −10°, a solution of 1.0 ml of anhydrous stannic chloride in 6 ml of carbon tetrachloride was added dropwise with stirring. After stirring 40 min at −10°, the mixture was poured into concentrated sodium bicarbonate solution and stirred for 1 hr until all gummy material was dissolved. The organic layer was separated, dried, and concentrated and the residual oil chromatographed on Florisil, eluting first with benzene, then with chloroform. Concentration of the combined chloroform fractions gave 0.80 g of reddish oil, which was hydrolyzed by 1.5-hr reflux under nitrogen with 12 ml of 10% sodium hydroxide. The solution was cooled, washed with ether, acidified with dilute sulfuric acid, and extracted with two 30-ml portions of ether. Concentration of the dried extracts left a solid residue, which was recrystallized from water. α-Indolmycenic acid (II) was isolated as a pale yellow solid: mp 175–176°, $[\alpha]^{25}_D$ +7.80° (*c* 2, CH_3OH); lit.[3] mp 181–182°, $[\alpha]^{25}_D$ −10° (*c* 2, CH_3OH). The infrared and nmr spectra were identical with those of the (−) acid.[14]

α-**(3-Indolyl)propionic Acid (VI).**—The racemic acid VI was prepared both by hydrolysis[7a] of α-(3-indolyl)propionitrile, itself prepared by the reaction of the indole Grignard reagent with α-bromopropionitrile, or by the base-catalyzed reaction of indole with lactic acid;[15,16] the latter method is far more practical. In a typical run, a 300 ml Monel autoclave was charged with 58 g of indole, 59 g of 85% aqueous lactic acid, and 45 g of 85% potassium hydroxide. The mixture was heated at 220° for 18 hr, cooled to 90°, and taken up in 250 ml of water. After washing with ether the alkaline solution was acidified to pH 1 with 12 *N* hydrochloric acid and extracted with ether. The extracts were washed with water, dried, and concentrated, leaving an oil which crystallized on standing. Recrystallization from chloroform gave 60.4 g (64%) of colorless solid, mp 105–106° (lit.[7b] mp 111–112°).

Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.55; H, 5.82; N, 7.41. Found: C, 69.37; H, 5.90; N, 7.32.

The acid was resolved with brucine according to the procedure of Sjöberg.[6b] (+)-VI had mp 136–137°, $[\alpha]^{23}_D$ 106° (benzene); lit.[5b] mp 138–140°, $[\alpha]^{23}_D$ 106.2° (benzene).

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(15) H. E. Johnson and D. G. Crosby, *J. Org. Chem.*, **28**, 1246 (1963).

(16) We are grateful to Dr. B. Franko-Filipic and Mr. W. McCarthy, FMC Corporation, Princeton, N. J., for their help in running large-scale preparations.

2-(3-Indolyl)propan-1-ol (V). A.—Racemic V was prepared by lithium aluminum hydride reduction of methyl α-(3-indolyl)-propionate (prepared from (±)-VI and diazomethane) in tetrahydrofuran. Work-up gave (±)-V as a pink, viscous oil, bp 155° (0.1 mm). The *p*-nitrobenzoate melted at 117°.

Anal. Calc for $C_{18}H_{16}N_2O_4$: C, 66.65; H, 4.97; N, 8.64. Found: C, 66.66; H, 5.05; N, 8.59.

B.—A solution of 3.7 g of (+)-VI in tetrahydrofuran was reduced with 40 ml of a 1 *M* solution of diborane in tetrahydrofuran at room temperature for 2.5 hr. The mixture was poured onto ice and saturated with salt. The organic layer was washed with 5% sodium bicarbonate, dried, and distilled, yielding (+)-2-(3-indolyl)propan-1-ol:[17] bp 135° (0.1 mm); lit.[17] bp 145° (0.15 mm), $[\alpha]^{22}D$ +28.1° (*c* 2.5, CH_3OH).

C.—Following the procedure of Schach von Wittenau and Els,[3] 120 mg of (+)-α-indolmycenic acid, $[\alpha]D$ +7.8°, was reduced with lithium aluminum hydride in ether, and the resulting glycol (III) cleaved with sodium periodate. The crude α-(3-indolyl)-propionaldehyde(IV) showed carbonyl absorption at 1720 cm^{-1}. The aldehyde was not further characterized, but reduced directly with lithium aluminum hydride in ether. After the usual work-up, 55 mg of (+)-V was obtained, $[\alpha]^{25}D$ +20° (*c* 2.7, CH_3OH), which showed tlc behavior and infrared spectra identical with those of the alcohol obtained in parts A and B. The alcohols from both parts B and C showed plain positive ORD curves from 300–600 nm.

(17) R. A. Robinson, U. S. Patent 2,908,691; *Chem. Abstr.*, **56**, 3455 (1962).

Registry No.—(+)-II, 25834-21-3; (±)-V, 25834-22-4; (±)-V *p*-nitrobenzoate, 25834-23-5; (−)-VIII, 25834-24-6; (−)-VIII Ag salt, 25834-25-7; indolmycin, 23369-88-2.

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Synthesis of D-Dihydrosphingosine[1]

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A stereospecific synthesis of D-dihydrosphingosine is recorded. The reaction of 6-benzyloxycarbonylamino-2,2-dimethyl-5-formyl-3a,5,6,6a-tetrahydro-D-ribofuro[2,3-*d*]dioxolane (**6**), prepared in three steps from 3-amino-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (**3**), with the Wittig reagent, prepared from tetradecyltriphenylphosphonium bromide (**2**) is described. The product, a mixture of cis and trans olefins (**8**), was deacetonated, and the resulting glycol was cleaved with sodium metaperiodate and then reduced with sodium borohydride to give crystalline 2-benzyloxycarbonylamino-D-*erythro*-Δ⁴-octadecene-1,3-diol (**11**). Hydrogenation of **11** over palladium on charcoal reductively deblocked the amine and saturated the olefin to give D-dihydrosphingosine (**12**).

D-Sphingosine (2-amino-1,3-dihydroxy-D-*erythro*-octadec-4-ene) and D-dihydrosphingosine (2-amino-1,3-dihydroxy-D-*erythro*-octadecane) are bases which serve as the backbone for the structures of cerebrosides, gangliosides, sphingomyelin, etc. Abnormal amounts of cerebroside derivatives have been observed in leukodystrophy,[2] Niemann–Pick and Tay–Sachs diseases,[3] etc. Unusual concentrations of sphingomyelin have been found in cataracts.[4]

Evidence is accumulating that sphingosine and dihydrosphingosine derivatives can act as prophylactics against certain laboratory induced diseases in animals. Thus intradermal injections containing cerebrosides offered significant protection against experimental allergic encephalomyelitis in rabbits.[5] Injection of ganglioside–cerebroside complexes offered relief from the symptoms of tetanus toxin in mice.[6] The suggestion was made[6] that such a technique might be of prophylactic value in human tetanus.

Biochemical studies using sphingosine derivatives isolated from natural sources were made difficult by the questionable purity of such materials. The syntheses of sphingosine[7] and dihydrosphingosine[8] reported have inevitably led to racemic mixtures which must then be resolved in order to obtain the desired optically active material.

Carbohydrates offer a wide assortment of extensively functionalized starting materials with known absolute configuration. By the attachment of a long alkyl chain to the appropriate amino sugar, the synthesis of optically pure sphingosine derivatives and analogs becomes a relatively simple procedure. A series of papers by Gigg, *et al.*,[9] describes the use of a Wittig condensation of an amino sugar derived from glucosamine with the ylide prepared from triphenylphosphine and tridecyl bromide to give D-phytosphingosine(4-hydroxydihydrosphingosine). The absence of a double bond in phytosphingosine circumvented the problem of cis *vs.* trans isomers of the Wittig product. For this same reason, the synthesis of D-dihydrosphingosine by a Wittig condensation was investigated initially and is reported here.

A logical starting material for the synthesis of D-dihydrosphingosine (and D-sphingosine) is the readily available 3-amino-3-deoxy-1,2:5,6-di-O-isopropylidene-

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