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ISOLATION AND ABSOLUTE CONFIGURATION OF INDOLMYCENIC ACID, AN INTERMEDIATE IN THE BIOSYNTHESIS OF INDOLMYCIN BY STREPTOMYCES GRISEUS.

U. Hornemann, L. H. Hurley, M. K. Speedie and H. G. Floss

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Indiana 47907

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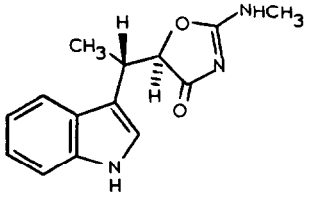
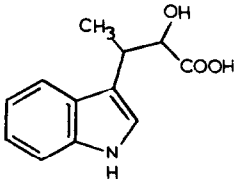
During our studies on the biosynthesis of indolmycin (IM) (I) by Streptomyces griseus (1) we have obtained evidence that indolmycenic acid (IMA) (II) [2-hydroxy-3-(3'-indolyl)-butyric acid] (2) is a precursor of this antibiotic (3). We here wish to report upon the isolation of this acid from the culture broth of S. griseus and the determination of its absolute configuration. We also report upon feeding experiments which further support the role of IMA as an intermediate in the biosynthesis of indolmycin.

Streptomyces griseus (ATCC 12648) was grown for 2 days in 100 ml shake cultures at 24° in a medium (4) containing soybean meal and distiller's solubles. The filtered broth was acidified with tartaric acid and extracted with ethyl acetate. The ethyl acetate phase was extracted with a 10% solution of sodium carbonate to remove the acidic substances. The latter phase was acidified with tartaric acid and re-extracted with ethyl acetate. Aliquots of this extract were subjected to tlc, on silica gel G, either directly [system A: chloroform-acetic acid 19:1, run six times, R_F α-IMA (2RS,3SR) 0.27, β-IMA (2RS,3RS) 0.37; system B: ethyl acetate-isopropanol-conc. ammonia 9:7:4, R_F α- and β-IMA 0.45] or after esterification with diazomethane [system C: chloroform-ethanol 5:1, R_F α- and β-IMA methyl ester 0.55; system D: ligroin-isopropanol 9:1, run four times, R_F α-IMA methyl ester 0.32, β-IMA methyl ester 0.38]. In all four systems a van Urk (6) positive spot in the extract corresponded in R_F to the position of authentic reference α-IMA (5) and reference α-IMA methyl ester respectively. Natural IMA was purified as its methyl ester by successive tlc in systems C and D. The mass spectrum of the methyl ester showed a molecular ion at m/e 233 and a fragmentation pattern identical to that of authentic synthetic IMA methyl ester.

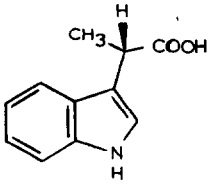
The absolute configuration of IMA was determined by chemical correlation with (S)-(-)-indoleisopropionic acid (7). Radioactive IMA ($9.0 \cdot 10^5$ dpm ^{14}C) obtained from a feeding experiment with DL-tryptophan-(alanine-3-^{14}C) was diluted with 5 mg of synthetic carrier material and reduced to the 1,2 diol by lithium aluminum hydride. The diol upon periodate cleavage produced β-methylindoleacetaldehyde which was oxidized with moist silver oxide to indoleisopropionic acid (III) ($8.8 \cdot 10^3$ dpm). Repeated co-crystallization of the indoleisopropionic acid obtained with the (S)-indoleisopropionic acid cinchonine salt showed no significant drop in specific activity (calc.: 59 dpm/mg, found: 1st cryst. 55 dpm/mg, 2nd cryst. 54 dpm/mg, 3rd cryst. 56 dpm/mg). In a previous experiment it had been confirmed that radioactive (R)-indoleisopropionic acid does not co-crystallize with the cinchonine salt of (S)-indoleisopropionic acid. Therefore, the absolute configuration at carbon atom 3 of natural IMA is S. The relative configurations of α - and β-IMA had previously been established (5) by stereoselective synthesis and N.M.R. studies as 2RS,3SR and 2RS,3RS, respectively, and the absolute configuration of natural IMA is, therefore, 2R,3S. The same absolute configuration has also been determined for indolmycin (8).



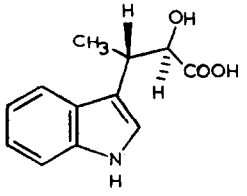
I Indolmycin



II Indolmycenic acid



III (S)-Indoleisopropionic acid



Natural indolmycenic acid

Naturally occurring IMA biogenetically labelled from DL-tryptophan-(alanine-3-^{14}C) was significantly better incorporated into IM than a mixture of α- and β-IMA obtained by hydrolysis of IM (Table I). In a parallel experiment, IM biosynthetically labelled from DL-tryptophan-(alanine-3-^{14}C) was not incorporated into IMA, indicating that the latter is not a degradation product of IM. Finally, all of the isomers of IMA were prepared in labelled form. The 2R,3R and 2S,3R isomers were prepared from (2RS,3SR)-β-methyltryptophan-(ring-^{3}H) by reaction with L-amino acid oxidase and catalase and subsequent $NaBH_4$ reduction of the (3R)-β-methylindolepyruvate formed. (2S,3S)- and (2R,3S)-IMA were prepared by hydrolysis of indolmycin labelled biosynthetically with DL-tryptophan-(alanine-3-^{14}C). Separation of each pair of isomers was carried out chromatographically in system A. An aliquot of each isomer was converted to the methyl ester and chromatographed in system D to check the radiochemical purity. Only the isomer corresponding to the naturally occurring IMA was significantly incorporated into IM (Table II).

Table I.

Precursor	Incorporation into IM (%)	Incorporation into IMA (%)
Natural IMA-(methylene-^{14}C)	28%	n.d.
Mixture of α- and β-IMA-(methylene-^{14}C)	12%	n.d.
IM-(methylene-^{14}C)	70% *	< 1.5%

n.d.: not determined * percent recovery

Table II.

Precursor	Incorporation into IM (%)
2R,3S-Indolmycenic acid	17%
2S,3S-Indolmycenic acid	< 0.1%
2R,3R-Indolmycenic acid	< 0.1%
2S,3R-Indolmycenic acid	< 0.1%

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