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Indole-3-Acetic Acid and 2-(Indol-3-ylmethyl)Indol-3-yl Acetic Acid in the Thermophilic Archaebacterium *Sulfolobus acidocaldarius*

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Indole-3-acetic acid (IAA) and 2-(indol-3-ylmethyl)indol-3-yl acetic acid were identified in lipid extracts of *Sulfolobus acidocaldarius*; they occurred at concentrations of 0.57 and 0.59 μmol/g (dry weight), respectively. The amount of IAA found in these cells is more than a thousand times greater than that found in a typical extract of a plant in which IAA serves as a plant growth hormone. Neither of these compounds was detected in the other archaebacteria that were analyzed; these included *Sulfolobus sulfataricus*, *Halobacterium salinarium*, and several strains of methanogenic bacteria. This is the first report of the natural occurrence of 2-(indol-3-ylmethyl)indol-3-yl acetic acid.

The archaebacteria have been shown to contain many unique compounds (3). These include methanopterin, methanofuran, coenzyme M, F_{430}, F_{420}, component B, the isopranoid lipids (3), and the recently characterized sulfohalopterin-2 from the halobacteria (4a). During a survey of the distribution of component B among members of the archaebacteria, I discovered that *Sulfolobus acidocaldarius* contains a relatively high amount of the indole-containing compounds indole-3-acetic acid (IAA) and 2-(indol-3-ylmethyl)indol-3-yl acetic acid (Fig. 1). Although IAA has been identified in several bacterial and plant sources (6, 7), this is the first report of the isolation of 2-(indol-3-ylmethyl)indol-3-yl acetic acid from a natural source.

S. acidocaldarius ATCC 33909 was grown for 3 days at 72 to 74°C in plastic carboys, each containing 18 liters of a medium consisting of 1.3 g of $(NH_4)_2SO_4$, 280 mg of KH_2PO_4, 250 mg of $MgSO_4 \cdot 7H_2O$, 75 mg of $CaCl_2 \cdot H_2O$, and 1.0 g of yeast extract per liter of water. The cultures were stirred by the addition of air at a rate of 0.3 liter/min through three glass frits mounted at the bottom of the bottle. The cells were isolated from the medium by membrane filtration followed by centrifugation (10,000 × *g*, 15 min). The yield of cells was 0.8 g (wet weight)/liter of medium. The lipids were extracted from the cells three times with a volume of a methylene chloride-methanol (1:1, vol/vol) equal to five times the wet weight of the cells in grams; the insoluble cell debris was separated from the extracting solvents by centrifugation. To the combined extracts was added a volume of 0.01 M HCl equal to half the total volume of the extract, and, after shaking, the methylene chloride layer was removed, dried with sodium sulfate, and concentrated by evaporation with a stream of nitrogen gas to a volume of about 1 ml. The indole-containing acids were extracted from this concentrated methylene chloride solution with 1 M sodium hydroxide (1 to 3 ml). After removal of the aqueous layer and acidification with 6 M HCl, the indole-containing acids were extracted back into methylene chloride. The acids, dissolved in methanol, were converted into their methyl esters by a brief treatment with a solution of diazomethane in diethyl ether.

Gas chromatography-mass spectrometry of this purified extract, separated on a glass column (0.3 × 183 cm) containing 3% OV-1 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefont, Pa.) programmed from 110°C at 10°C/min, gave only two major peaks, A and B. The mass spectrum of the first peak, A, showed major ions at *m/z* 189 (M^+) (33.5% of base peak) and *m/z* 130 ($M^+ - 59$) (base peak). The odd mass of the molecule ion indicated that the compound contained an odd number of nitrogen atoms. The mass spectrum of peak A, and its gas chromatographic retention time on two different columns, was found to be identical to that obtained from a known sample of the methyl ester of IAA. Peak A had the same R_f on thin-layer chromatography on silica gel 60 F-254 (E. Merck AG, Darmstadt, Federal Republic of Germany) in three different solvent systems (R_f = 0.075 in benzene, 0.097 in hexane-diethyl ether [7:3, vol/vol], and 0.22 in methylene chloride) as a known sample of the methyl ester of IAA; a sample of the material purified by preparative thin-layer chromatography showed λ_{max} in ethanol at 224, 283, and 291 nm, thereby confirming the presence of the indole nucleus in the molecule.

The mass spectrum of the second peak, B, showed a molecular ion at *m/z* 318 (M^+) (99.0% of the base peak). Structurally important fragment ions at *m/z* 259 ($M^+ - 59$) (82.0% of the base peak) and *m/z* 245 ($M^+ - 73$) (base peak) were also observed. Accurate mass measurements showed the molecular ion to have a mass of 318.1367, corresponding to $C_{20}H_{18}O_2N_2$ (error = −0.1 millimass unit), and showed the fragment ions to correspond to the loss of $C_2H_3O_2$ ($COOCH_3$) and $C_3H_5O_2$ (CH_2COOCH_3), respectively, from the molecular ion. A sample of the material purified by preparative thin-layer chromatography showed λ_{max} in ethanol at 225, 283, and 291 nm, thereby indicating the presence of at least one indole ring.

The high degree of unsaturation in this compound, as well as the presence of two nitrogens, strongly supported the idea that the molecule contained two indole rings. If correct,

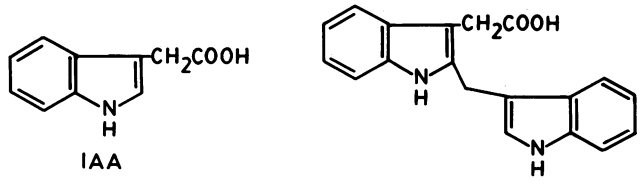

FIG. 1. Structures of IAA and 2-(indol-3-ylmethyl)indol-3-yl acetic acid.

then, on the basis of the elemental formula of the molecule and the observed fragmentation of the methyl ester, there were only two possible structures, the methyl ester of either 3,3-bisindol-3-yl propionic acid or 2-(indol-3-ylmethyl)indol-3-yl acetic acid.

Comparison of the gas chromatographic retention time and mass spectrum of the methyl ester of the compound isolated from *S. acidocaldarius* with those of the methyl esters of these known compounds clearly showed only 2-(indol-3-ylmethyl)indol-3-yl acetic acid to be the same as the natural product. This was further confirmed in that both the natural and synthetic materials had the same R_f on silica gel 60 F-254 in three different solvent systems (R_f = 0.050 in benzene, 0.054 in hexane-diethyl ether [7:3, vol/vol], and 0.19 in methylene chloride), and both materials had the same UV absorption spectrum. 2-(Indol-3-ylmethyl)indol-3-yl acetic acid was prepared by the condensation of 3-hydroxymethylindole with IAA (1), and 3,3-bisindol-3yl propionic acid was prepared by reaction of indole with propiolic acid (9).

Quantitation of the indoles isolated from the cells was accomplished by absorbance spectroscopy of samples purified by thin-layer chromatography. This analysis showed that the cells contained 0.57 μmol of IAA per g (dry weight) and 0.59 μmol of 2-(indol-3-ylmethyl)indol-3-yl acetic acid per g (dry weight). If we assume that 50% of the cell pellet weight is cytoplasm, then the concentration of these materials in the cells would be about 0.3 mM. Neither of these compounds was detected by gas chromatographic-mass spectrometric analysis of extracts of *Sulfolobus solfataricus* grown and extracted under the same conditions as described above for *S. acidocaldarius*. The compounds were also not detected by gas chromatography-mass spectrometry in extracts of *Methanobacterium formicicum*, *Methanobacterium smithii*, *Methanobrevibacter* sp. strain 10-16B, or *Halobacterium salinarium*.

The occurrence of these indole compounds in *S. acidocaldarius*, but not in *S. solfataricus*, indicates a clear metabolic difference between these two species of extremely thermophilic archaebacteria. This metabolic difference is further supported by the fact that *S. solfataricus* contains solfapterin, a recently characterized cationic pterin, which is not found in *S. acidocaldarius* (X. Lin and R. H. White, unpublished data). The reported occurrence of large amounts of indole compounds in members of the archaebacteria is not unprecedented, however, since indole has been reported to occur throughout the extremely halophilic archaebacteria (4).

IAA is known to occur widely in small amounts (1 to 100 μg/kg [fresh weight]) in the tissues of higher plants (6, 7), where it functions as a plant auxin. These concentrations, however, are at least 1,000-fold less than those found in *S. acidocaldarius*, indicating that the IAA must serve a different function in these bacteria from the one it serves in plants. An indication of this function may be inferred from the fact that 2-(indol-3-ylmethyl)indol-3-yl acetic acid, which is known to be produced by the peroxidase-catalyzed oxidation of IAA (8), was identified along with IAA. Since this reaction consumes oxygen (2), IAA oxidation could serve as a primitive system to reduce the concentration of toxic oxygen in the cells. Even in the absence of a peroxidase system, the autoxidation of indoles is still significantly rapid enough for them to function as an oxygen-scavenging system, especially considering the high temperatures at which these bacteria grow (5).

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