BIOCHEMICAL LETTERS

Dicyclohexylamine is not an inhibitor of spermidine synthase

It was reported by Hibasami *et al.* (1980) that dicyclohexylamine is a potent inhibitor of spermidine synthase (EC 2.5.1.16) from rat ventral prostate, with an IC_{50} of about $8 \mu M$. The inhibition is competitive with respect to putrescine with a K_i value of $0.2 \mu M$. This work was extended to demonstrate antitumour effects of dicyclohexylamine against Ehrlich ascites carcinoma, Ehrlich solid carcinoma, sarcoma-180 and P388 leukaemia in mice, which is accompanied by reduced levels of spermidine in the tumour cells (Hibasami & Ito, 1981; Ito *et al.*, 1982). Other workers have found that dicyclohexylamine inhibits spermidine synthase and growth of various organisms, including *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus uberis* (Pegg *et al.*, 1983; Bitonti *et al.*, 1982; Mattila *et al.*, 1984) and that growth inhibition is reversed by addition of spermidine (Mattila *et al.*, 1984).

Hibasami *et al.* (1980) also noted that dicyclohexylamine is a more potent inhibitor of spermidine synthase than is cyclohexylamine. This observation was supported by Bitonti *et al.* (1984) who found that dicyclohexylamine inhibits spermidine synthase from *Trypanosoma brucei* by 50% at $3 \mu M$, whereas the IC_{50} for cyclohexylamine was reported to be $15 \mu M$. Similar findings were reported by Sindhu & Cohen (1984) using spermidine synthase from leaves of the Chinese cabbage *Brassica pekinensis* cultivar Pak Choy. The dicyclohexylamine used in all these studies was obtained from the Sigma Chemical Co.

In the present communication we report studies of spermidine synthase inhibition by dicyclohexylammonium sulphate (Sigma), dicyclohexylamine free base (Fisons), dicyclohexylammonium sulphate (prepared in-house), cyclohexylamine free base (BDH) and cyclohexylammonium phosphate (Sigma). Spermidine synthase in crude cytosol extracts of cultured human skin fibroblasts was assayed by the methods of Raina *et al.* (1983) with 1.0 mM -putrescine and $20 \mu M$ -decarboxylated *S*-adenosylmethionine as substrates. Under such conditions dicyclohexylammonium sulphate (Sigma) inhibited the enzyme with an IC_{50} of $9.5 \mu M$ and cyclohexylamine, either as free base or phosphate salt, gave IC_{50} values of $12\text{--}14 \mu M$ (Fig. 1). However, dicyclohexylamine free base and its sulphate (prepared in-house) failed to inhibit spermidine synthase. The chemical name given to the Sigma compound misled us to believe that the compound was a salt of dicyclohexylamine. The anhydrous M_r of 296.4 is inconsistent with such a structure, but consistent with it being bis-cyclohexylammonium sulphate. Microanalysis of this material revealed that it possesses a C:N

Fig. 1. Effect of cyclohexylamine on spermidine synthase

$\square$, 'Dicyclohexylammonium sulphate' (Sigma); $\circ$, cyclohexylamine free base; $\bullet$, cyclohexylamine phosphate.

Table 1. Microanalysis of 'dicyclohexylammonium sulphate' (Sigma)

	Observed for 'dicyclohexylammonium sulphate' (Sigma)	Theoretical for bis-cyclohexylammonium sulphate ($C_{12}H_{28}N_2SO_4$)
C	48.7%	48.6%
H	9.7%	9.5%
N	9.4%	9.5%
S	10.8%	10.8%

ratio of 6:1 and a S:N ratio of 1:2 (Table 1), confirming its identity as bis-cyclohexylammonium sulphate.

Furthermore, reversed-phase h.p.l.c. analysis demonstrated that the Sigma compound co-chromatographs with cyclohexylamine.

We suggest, therefore, that 'dicyclohexylammonium sulphate' (Sigma) be given its correct chemical name, bis-cyclohexylammonium sulphate, and that the scientific literature referring to the ability of dicyclohexylamine to inhibit spermidine synthase is due to cyclohexylamine, but at twice the molarity quoted.

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