3-Ethyl-8-methyl-1,3,8-triazabicyclo[4,4,0]decan-2-one: a new antifilarial agent*

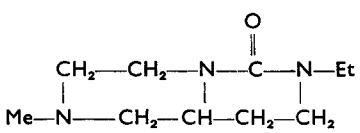
Earlier studies on the antifilarial activity of some open chain (Sewell & Hawking, 1950; Wadia, Asthana & others, 1958) and cyclic (Reinertson & Thomas, 1955; Wadia & Anand 1958a, b; Brookes, Terry & Walker, 1957; Nagpal & Dhar, 1965) analogues of diethylcarbamazine (Hetrazan) have shown that any modifications to the piperazine ring led to a lowering or loss of antifilarial activity. In further exploration in this field 3-ethyl-8-methyl-1,3,8-triazabicyclo[4,4,0]decan-2-one (I) has been synthesized† and found to possess high microfilaricidal activity in cotton-rats

Table 1. *Comparative efficacy of Compound I and diethylcarbamazine against* L. carinii *infection in cotton rat*

Compounds as base	ED90* (mg/kg) × 6 days, cotton rats i.p.	oral	LD50 (mg/kg) in mice i.p.	oral	Microfilarial count as % of pre-treatment level after stopping treatment on days: 1	8	Therapeutic index
Diethylcarbamazine	6	–	240	–	10	80–95	40
I	1	2	300	600	8	27–30	300

* The dose which when administered would clear 90% of the pretreatment circulating microfilariae.

infected with *Litomosoides carinii*. Screening was by the technique of Hawking & Sewell (1948). The comparative efficacy of I and of diethylcarbamazine is described in Table 1. Compound I thus seems to be significantly more effective than diethylcarbamazine in reducing the microfilariae in cotton rats. Its activity also seems to persist longer than diethylcarbamazine. Like diethylcarbamazine it was without significant action against the adult worms of *L. carinii*. Prolonged treatment for 21 days at 25 mg/kg intraperitoneally twice daily killed only about 10–20% of the adult worms.



I

Compound I was effective against the infective larvae of *L. carinii* in cotton rats. When cotton rats were treated with 25 mg/kg of the compound daily for three consecutive days commencing immediately after exposure to infected mites (*L. bacoti*) in desiccators for 24 h they failed to show any microfilariae or adult worms up to 90 days after exposure. Untreated controls invariably had adult worms and microfilariae.

Division of Medicinal Chemistry,

(Miss) RANJANA SAXENA
R. N. IYER
NITYA ANAND

* Communication No. 1393 from the Central Drug Research Institute, Lucknow, India.

† U.S. and Indian patent application pending.

Division of Chemotherapy,
Central Drug Research Institute,
Lucknow (U.P.), India.

R. K. CHATTERJEE
A. B. SEN

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The effect of ovariectomy on the γ-aminobutyric acid content in the cerebral hemispheres of young rats

The removal of some endocrine glands affects the γ-aminobutyric acid (GABA) content in the rat brain. The GABA content is decreased after castration, but returns to normal after injection of testosterone propionate (Tzu-Yu Li & Chang-Hua Wu, 1964). Adrenalectomy results in decreased GABA content of rat brain (Rindi & Ventura, 1961), cerebral cortex (Vernadakis & Woodbury, 1959; Pandolfo & Macaione, 1964), and subcortex (Sutherland & Rikimaru, 1964). Thyroidectomy also decreased the brain GABA content (Nishioka, 1960). I have examined the effect of ovariectomy on the GABA content in the cerebral hemispheres of young rats.

Young female rats, 40–48 g, were divided into 4 equal groups of 9 each. One group received no treatment. The other animals were ovariectomized and left for 2, 15 and 30 days before being killed by guillotine for the estimation of GABA content in their cerebral hemispheres. At that time the rats weighed 60–70 g. For the analysis the cerebral hemispheres of three animals were pooled.

GABA was quantitatively determined using a chromatographic and colorimetric method. Within one min of death the brain was isolated and the cerebral hemispheres were separated and frozen to −4 to −6°. The frozen pooled sample was quickly weighed, triturated to a homogeneous mixture, and 60 ml of ethanol 75% was used as a solvent. The mixture was centrifuged and the supernatant fluid was evaporated to dryness. The residue was cooled and dissolved in distilled water. This extract was centrifuged and an amount of cerebral hemisphere extract equivalent to 60 mg of the original wet tissue was applied by an Agla micrometer syringe to a 20 × 46 cm band of chromatographic paper.* The chromatogram was developed with the descending technique by the one dimensional method using the solvent phenol–water (4 : 1 v/v) for 18 to 20 h, after which the solvent was removed. The paper was sprayed on both sides with 0·1% ninhydrin in butanol and left suspended for 30 min at 93° for maximal colour development (Roberts & Frankel, 1950). The GABA spots were eluted by distilled water, and the extinction read in a Unicam SP1300 colorimeter using filter No. 4. The relation between the extinction and the concentration of pure GABA† was determined under the experimental conditions. The formula of the curve was obtained by the least square method (Waugh, 1952) and used to convert the colorimetric readings into the equivalent concentrations of GABA.

The relation between different amounts of an authentic sample of GABA when used in between 5 and 35 μg, with the extinction of the ninhydrin-stained chromatographic spots had the general formula $Y = 0{\cdot}011 + 0{\cdot}0051\ X$.