# 1,1,3-TRICYANO-2-AMINO-1-PROPENE (U-9189), A BIOLOGICALLY-ACTIVE COMPONENT OF AQUEOUS SOLUTIONS OF MALONONITRILE

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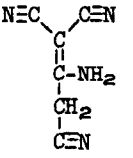
Malononitrile has been reported to increase the nucleoprotein content of neurons selectively in the rabbit (Hyden and Hartelius, 1948). It was demonstrated subsequently that the malononitrile used in these experiments contained an ultraviolet-absorbing material ($\lambda$ max = 272 mμ) other than malononitrile, and that samples of malononitrile from other sources developed this chromophore in aqueous solution (Mendelson et al., 1954). Since the unique cytological effects attributed to malononitrile might be due actually to the 272 mμ-absorbing material, the nature of this chromophore was investigated.

The rate of formation of the 272 mμ chromophore from malononitrile was base catalyzed. A 0.5% solution of malononitrile in 0.025 M borate buffer (pH 9.7) was incubated for 12 hours at 25°. The reaction mixture was acidified to pH 2.0, concentrated in vacuo, and partitioned between 1-butanol and 0.01 N hydrochloric acid by means of a Craig countercurrent apparatus. Analysis of the distribution data revealed a major symmetrical peak of constant absorptivity at 272 mμ. The solute in the peak, obtained by evaporation to dryness and recrystallization from water, yielded light yellow birefringent needles, $\lambda$ max = 274 mμ (0.01 N ethanolic sulfuric acid), log $\epsilon$ = 4.181, m.p. 170-172°, pK'a (57% ethanol) = 8.4. Anal. Calcd. for $C_6H_4N_4$: C,54.55; H,3.06; N,42.40; mol. wt., 132.1. Found: C,54.30; H,3.21; $N_K$,42.17; mol. wt. (Rast), 135; equiv. wt., 131.

That the crystalline material was 1,1,3-tricyano-2-amino-1-propene

(U-9189), a dimer of malononitrile, was indicated by the nuclear magnetic resonance spectrum, which revealed the presence in equal amounts of only amino and methylene hydrogen.



1,1,3-tricyano-2-amino-1-propene
(U-9189)

This structure was confirmed by comparison with material synthesized according to Carboni (1955). Both preparations were identical on the basis of elemental analyses, ultraviolet and infrared spectra, NMR spectra, m.p. and mixed m.p.

Recent experiments have shown that U-9189 elicits rather unique biochemical responses. Fifty per cent uncoupling of oxidative phosphorylation was produced by $2.5 \times 10^{-4}$ M U-9189 when α-ketoglutarate was used as substrate with rabbit liver mitochondria. In addition, U-9189 possesses marked antithyroid activity in rats (S. H. Ingbar; B. M. Jagiello, Personal Communications) and humans (S. H. Ingbar; W. E. Mayberry, Personal Communications). Of special interest is the observation that the increased ultraviolet absorption shown by neurons from malononitrile-treated rabbits (Hyden and Hartelius, 1948) also could be produced by U-9189 treatment. Moreover, analyses of single Deiter cells from U-9189-treated animals showed highly significant increases in concentrations of protein, ribonucleic acid, succinoxidase, and cytochrome oxidase (R. G. Grenell and H. Hyden, Personal Communication).

Since malononitrile solutions used by other investigators might have contained unknown amounts of U-9189, it is suggested that results of previous biological studies with malononitrile be re-evaluated. Details of this work and the biochemical properties of U-9189 will be presented subsequently.

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