Tetrahedron Vol. 29, pp 2743 to 2746 Pergamon Press 1973. Printed in Great Britain

THE STRUCTURE OF COMPOUND 593A, A NEW ANTI-TUMOR AGENT

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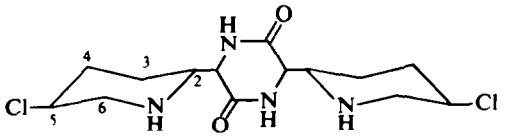
(*Received in the USA* 28 *March* 1973; *Received in the UK for publication* 17 *May* 1973)

Abstract—Compound 593A, a new anti-tumor agent, is believed to be 3,6-bis(5-chloro-2-piperidyl)-2,5-piperazinedione. Its structure was deduced solely from physical evidence, in particular from nuclear magnetic resonance and mass spectral observations.

The biological properties and reactions of Compound 593A, a new anti-tumor agent isolated from *Streptomyces griseoluteus*, have recently been disclosed.[1,2] Its structure, deduced largely from NMR and mass spectral observations, is believed to be 3,6-bis(5-chloro-2-piperidyl)-2,5-piperazinedione (1):



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The present report details the evidence leading to this structure proposal.

The microanalysis for Compound 593A hydrochloride corresponded to an empirical formula $C_7H_{11}ClN_2O \cdot HCl$. Solid state IR(Nujol) indicated the presence of NH and/or OH (3070, 3110 and 3210 cm^{-1}), NH^+ (2400–2700 cm^{-1}) and ketonic functionality, possibly monosubstituted amide (1685, 1665 cm^{-1}). Other than end absorption, the compound exhibited no UV absorption down to 220 nm.

The 100 MHz NMR spectrum (Fig 1a) suggested a cyclic structure because of the variety of coupling constants and the moderate line broadening evident in many of the signals. Spin-decoupling studies established a number of vicinal relationships but it was apparent that structure proposals at this stage would be premature in view of the overall complexity of the spectrum. Quite clearly, it would be necessary to first identify the class of compound to which 593A belonged. This vital information was supplied by the NMR spectrum of 5-hydroxypipecolic acid (Fig 1b and Ref 3). Aside from the doublet at 4·58δ, the striking similarity of the two spectra warranted the conclusion that 593A was a 2,5-disubstituted piperidine in which both substituents were equatorial. The close correspondence of the chemical shifts further required that the substituents at C_2 and C_5 have similar influences

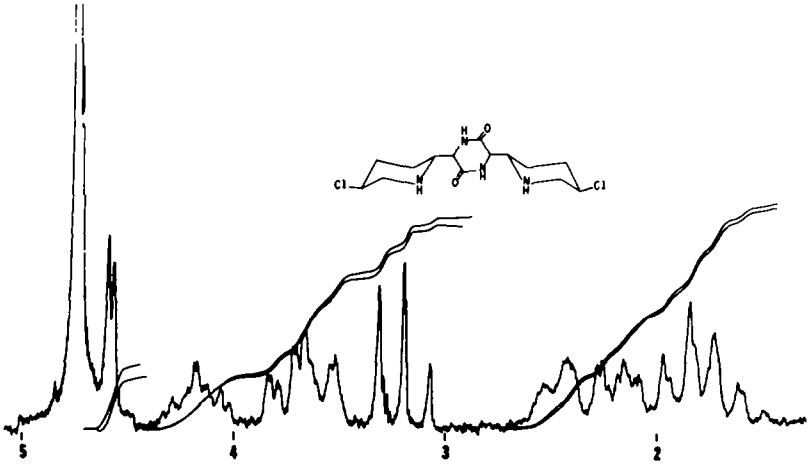


Fig 1a. NMR spectrum of 593A.

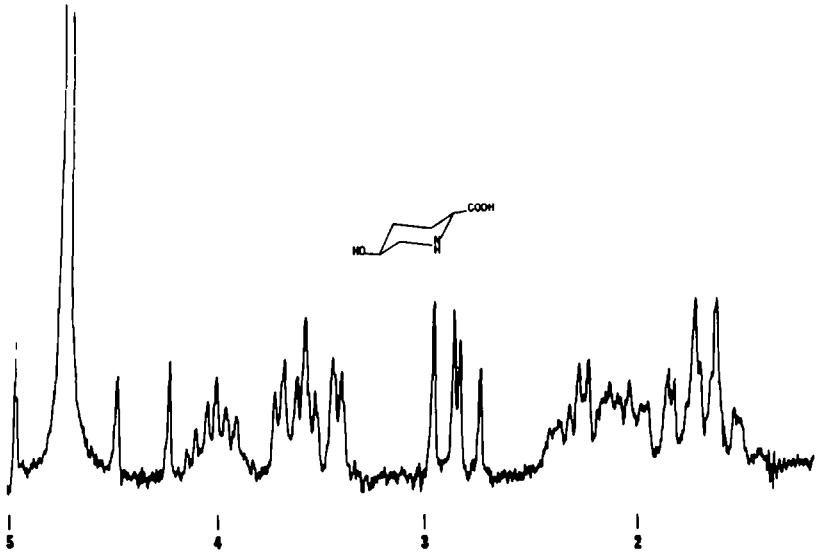
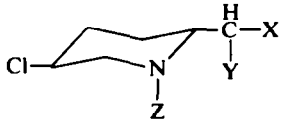


Fig 1b. NMR spectrum of 5-hydroxy pipecolic acid.

as a carboxy and hydroxy group respectively. Since an oxygen was apparently excluded at C_5 on the basis of IR and empirical formula considerations,† the chlorine was chosen as the most likely alternative. Spin-decoupling experiments established that the signal at 4·58δ represented a proton on a C atom which was attached to the 2 position of the piperidine ring. Based on these observations, the partial structure (2) was proposed wherein the second

†The empirical formula allowed only one oxygen which, from IR was part of a CO group. Although later mass spectral findings indicated that 593A was a larger entity than had been previously suspected, no revision in the above conclusion was necessary because of symmetry relationships.



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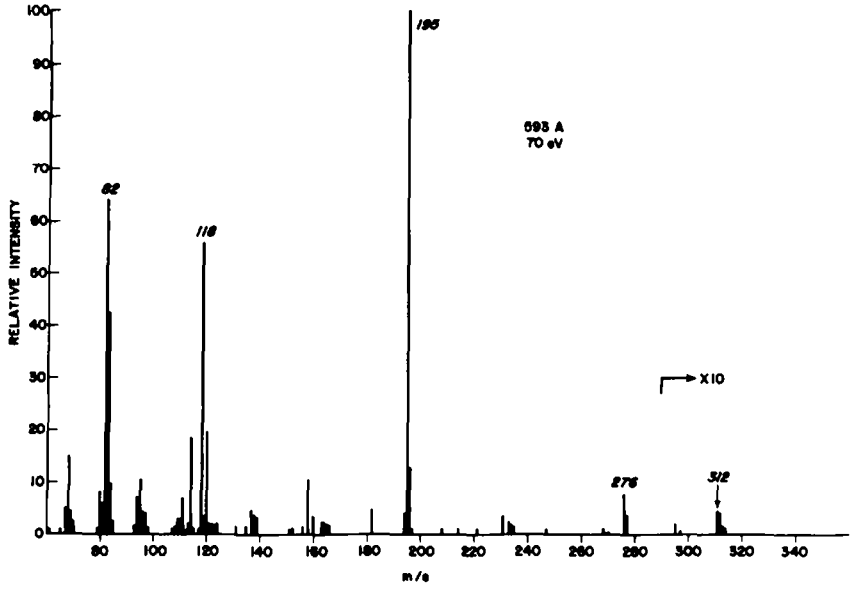
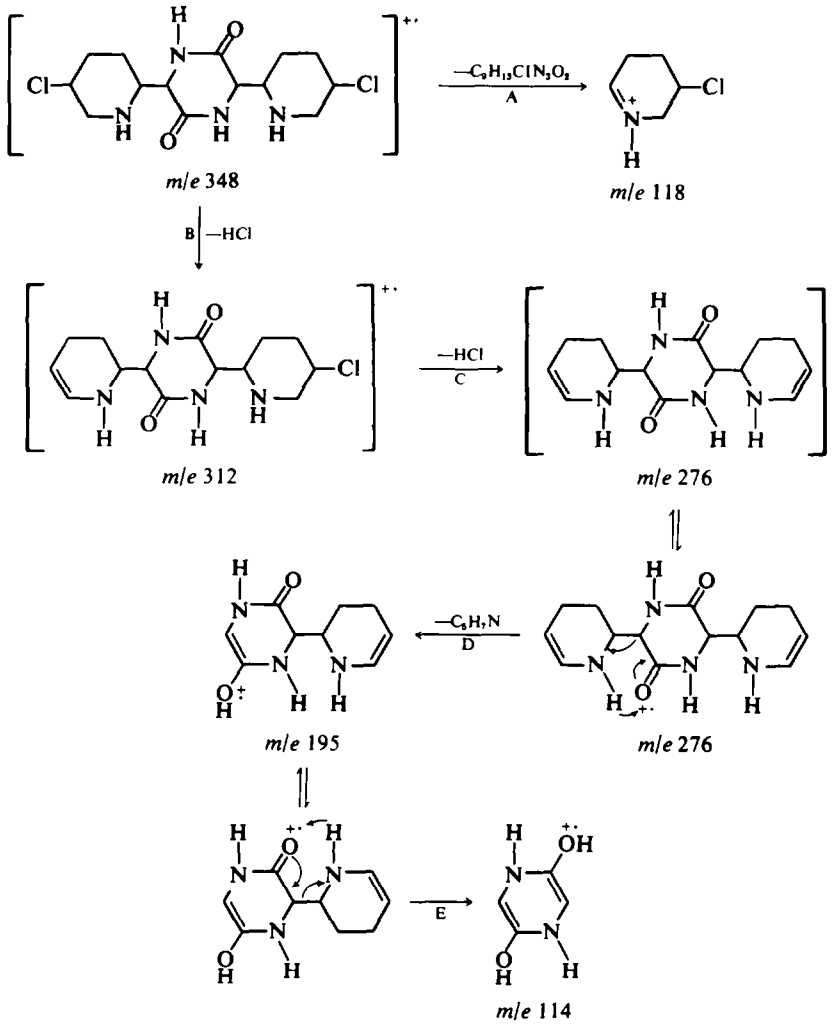


Fig. 2 Mass spectrum of 593A.



SCHEME 1

nitrogen and the CO group were part of the unidentified fragments.

Despite the fact that all non-labile hydrogens were now defined, unexpected difficulties were experienced in formulating structures consistent with the suggested empirical formula. The solution to this problem came from the analysis of the mass spectral data.

The low resolution mass spectrum of 593A (Fig 2) showed no molecular ion. A cluster of low-intensity peaks at *m/e* 312–315 exhibited a pattern compatible with the presence of one chlorine atom in the fragment. A sizeable peak, which does not contain chlorine, occurred at *m/e* 276. A high resolution mass measurement on this peak led to the proposal of $C_{14}H_{20}N_4O_2$ as the formula for this fragment ion (calcd 276·1586; found 276·1582). The presence of the chlorine containing peak at *m/e* 313 and the likelihood of an even number of nitrogen atoms in the molecule precluded the consideration of *m/e* 313 as the molecular ion. It must be either the $[M^{+\cdot}+1]$ ion or a fragment ion. This information, together with the microanalytical data, led to the view that the *m/e* 313 peak was a fragment ion. Apparently, this ion was formed from an unstable molecular ion of *m/e* 348 or *via* thermal degradation from the molecule $C_{14}H_{22}Cl_2N_4O_2$, mol wt 348.

The base peak of the mass spectrum occurred at *m/e* 195 and could arise from the lose of 2 HCl molecules plus one of the modified piperidyl rings *via* a McLafferty rearrangement (pathway B, C, D, Scheme 1). The intense peak at *m/e* 118, containing one chlorine atom, may be attributed to the intact piperidyl ring moiety resulting from homolytic cleavage of the bond joining this ring with the diketopiperazine ring (pathway A, Scheme 1), whereas the intense peaks at *m/e* 82 and 83 can result from further decomposition of the *m/e* 118

ion *via* loss of an HCl molecule or a chlorine atom, respectively. Finally, the prominent peak at *m/e* 114 may be visualized as arising from the equilibrium tautomer of the ion at *m/e* 195 *via* a second McLafferty rearrangement (pathway E, Scheme 1).

The key deduction from the mass spectral data, i.e., that the molecular weight was 348, necessitated thinking in terms of a structure containing two piperidyl moieties linked *via* a bridge in a symmetrical fashion. The nature of this bridge was partially defined by the missing fragment, $C_4H_4N_2O_2$, (deduced from the sum of the mass spectral data) a suspected amide group (IR) and the proton doublet at 4·58δ. The realization that all of these features could be accommodated by a diketopiperazinyl group then led directly to the proposal of 1 for 593A.

Since 593A has not been compared with a synthetic specimen of the 3,6-bis(5-chloro-2-piperidyl)-2,5-piperazinedione, the foregoing presentation does not meet all the classical requirements for a proof of structure. It is felt, however, that the spectroscopic evidence is sufficiently compelling so that the case for the proposed structure is not materially weakened.

EXPERIMENTAL

Mass spectra were obtained using a CEC 21-110 mass spectrometer. Operating conditions were: source temp, 210°C; ionizing current, 100 μA; electron energy, 70 eV; accelerating voltage, 8·12 kV. High resolution measurements were made *vis* the peak matching method. Sample introduction was through a direct probe inlet system.

The NMR spectrum was determined in D_2O (approximate concentration: 5% [w/v]) on a Varian HA-100D spectrometer. Hexamethyldisiloxane was used as an external reference and the data are expressed in ppm relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal reference. Assignments: δ 4·58 (d, J = 2·5, NCHC = 0); 4·18 (m, H_5[axial]); 3·8 (m, H_6[equatorial]); 3·6 (m, H_2[axial]); 3·19 (t, J = 12·0, H_6[axial]); $2·4_7$ (m, H_3[equatorial]); $2·1_8$ (m, H_4[equatorial favored]); 1·9 (m, H_4[axial favored]); 1·7 (m, H_3[axial]).

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