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CHARACTERIZATION OF TWO FLUORESCENT SUBSTANCES DERIVED FROM N^6-(Δ^2-ISOPENTENYL)ADENOSINE+

Girish B. Chheda,* Shib P. Dutta and A. Mittelman
General Clinical Research Center
Department of Surgery
Roswell Park Memorial Institute
Buffalo, New York 14203
and
Lubomyr Baczynskyj

The Upjohn Company
Kalamazoo, Michigan

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N^6-(Δ^2-Isopentenyl)adenosine (i^6A, I, Chart I)[1], the anticodon adjacent modified nucleoside present in $tRNA^{Ser}$[2], $tRNA^{Tyr}$[3] and in other tRNA's responding to the codons starting with U, has been found to undergo a slow transformation to three intensely fluorescent substances and adenosine. Since i^6A as a free nucleoside has been used in the clinic as an antileukemic agent[4] and as a part of the macromolecule it appears to participate in codon-anticodon interaction, it was of importance to elucidate the structures of these fluorescent substances derived from i^6A.

Two fluorescent compounds were isolated from a two year old batch of i^6A* (which was initially homogenous in paper and thin layer chromatography) by separation on a silica gel column and then repeated preparative paper chromatography and finally crystallized from ethanol. The major fluorescent compound (II, Chart I), m.p. 168-170° (dec.) showed its u.v. absorption maxima (Fig. IA) in 0.1 N HCl at 223 (ε: 25,100) and 276 nm (ε: 10,600); in water at 232 (ε: 30,300), 260 (sh), 268 (ε: 6,700), 278 (ε: 6,600) and 295-310 nm (plateau); in 0.1 N NaOH at 259 (sh), 268 (ε: 8,700), 278 (ε: 7,900) and 295-310 nm (plateau). The u.v. spectra were similar to those of the 3-alkylimidazo [2,1-i]purine derivatives.[5,6] Further evidence for the presence of 3-alkylimidazo[2,1-i]purine ring system was provided by the mass and NMR spectrum of II. The mass spectrum of II gave a highest mass ion at m/e 331 along with an intense peak at 18 presumably due to thermal dehydration of the compound II. The fragment ions at m/e 242, 229 and 199 can be assigned to the common purine nucleoside fragments M-89, B+30 and B_H respectively on the basis of new molecular ion m/e 331 formed by dehydration. In order to confirm that the m/e 331 ion was in fact due to a loss of the

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*The initially homogenous batch of i^6A on storage led to the formation of three fluorescent substances and adenosine. The quantitative analysis by paper chromatography of a two year old batch revealed that the major fluorescent compound (II) was present in 4.5%, minor fluorescent compound (IV) in 2.7%, the trace fluorescent material in less than 1% and adenosine in 2.7%.

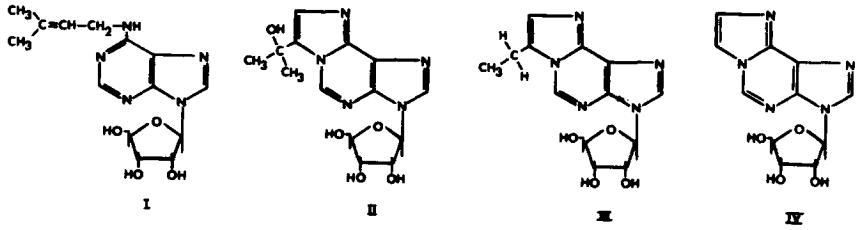
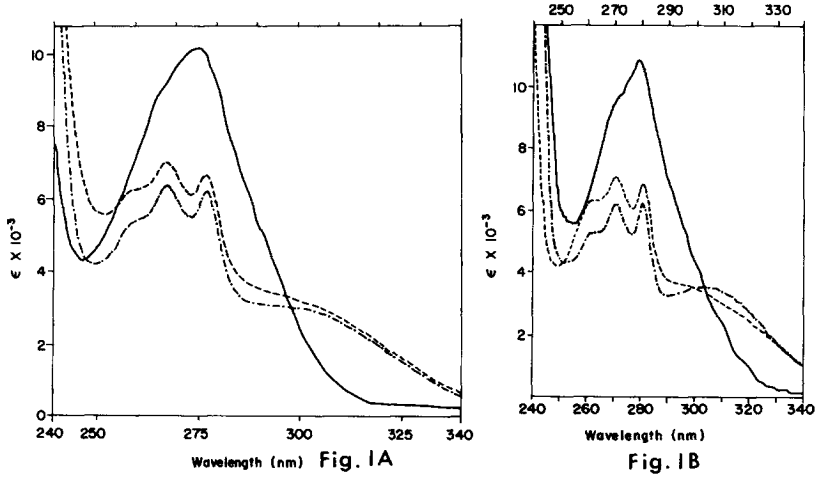


CHART I

elements of water, compound II was converted into a trimethylsilyl (TMS) derivative and the mass spectra were determined. It gave a molecular ion at m/e 637 which was in agreement with the molecular weight of tetra TMS derivative of compound II (Fig. II). The other fragment ions in the mass spectrum of the TMS derivative of II can be readily assigned on the basis of the general fragmentation pattern of TMS derivatives of purine nucleosides.[7] These results confirmed that the molecular weight of II was indeed 349 (331 + 18). Final confirmation for the 3-alkylimidazo[2,1-i] purine ring system in II was obtained from a comparison of the UV spectra of II with those (Fig. IB) of a synthetic model compound III (Chart I, Anal. $C_{14}H_{17}N_5O_4$.HBr, m.p. 132-34°) prepared by the reaction of adenosine with α-bromobutyraldehyde.* The NMR spectrum of II revealed peaks at δ 8.20 and 7.22 which could be assigned to the protons attached to C_5, C_2 & C_8 respectively. The NMR spectrum of compound III also confirmed these assignments. Hydrolysis of II with N/10 HCl at 100° liberated ribose, identified by paper chromatography. The presence of ribose was also



UV spectra of the compound II (Fig. IA) and compound III (Fig. IB); —— 0.1N HCl; -·-·-·water; - - - - 0.1 N NaOH.

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*Also synthesized by Prof. N.J. Leonard and his associates; Personal Communication.

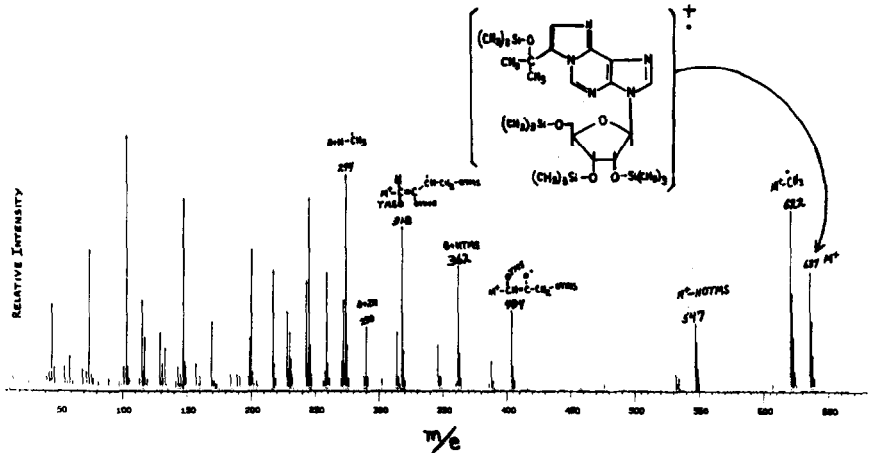

Fig. II. Mass Spectrum of Tetra TMS Derivative of Compound II

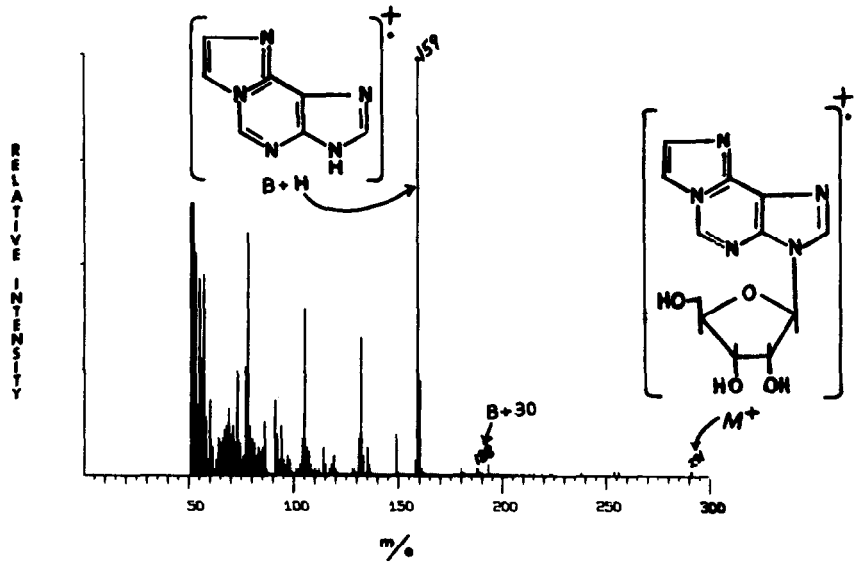

Fig. III. Mass Spectrum of Compound IV

confirmed by the peaks at 5.90δ and 3.78δ corresponding to the anomeric proton and 5'-CH_2 of the sugar moiety in the NMR spectrum of II. On the basis of these results, the major fluorescent compound has been assigned the structure II.

The minor fluorescent compound (IV, Chart I, m.p. 233-35° (dec.) also had UV spectra similar to those of II and III. The mass spectrum (Fig. III) of IV gave a highest mass ion peak at m/e 291 (M+) followed by peaks at m/e 188 (B+30) and m/e 159 (B+H). The mass spectrum of the TMS derivative

of IV gave the required molecular ion at m/e 507. The structure of IV was finally confirmed by its synthesis from adenosine and α-bromoacetaldehyde following the procedure of Barrio et al.[6] The UV and mass spectra and the mobilities in thin layer and paper chromatography of the synthetic material were identical to those of the fluorescent compound IV.

Mild oxidative cyclization of the isopentenyl side chain with an appropriately situated oxygen to form a dihydrofuran ring has earlier been reported.[8] It appears that a similar mechanism may explain the formation of II from i^6A. The initial step in this case would be the cyclization of the isopentenyl side chain in i^6A with the N^1-nitrogen to form an imidazoline ring which on aerial oxidation would furnish the imidazo purine derivative II. Compound IV can then arise from II by abstraction of a proton from the hydroxy group of the side chain followed by the elimination of acetone. In a preliminary study, we have been able to convert a pure sample of i^6A in aqueous ethanol (pH3) to compound II in about 3% yield along with a trace of IV. Attempts are in progress to develop the conditions for the quantitative conversion of i^6A into II. This conversion of i^6A (I) into the fluorescent substance (II) is of significance since under proper conditions it should be possible to convert i^6A to II in the intact molecule of t-RNA, where it could serve as a built-in fluorescent probe for studying the structure-function relationship in the t-RNA molecule. Preliminary studies indicate that in addition to i^6A, other t-RNA components like zeatin riboside containing this type of side chain also undergo similar transformations.

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