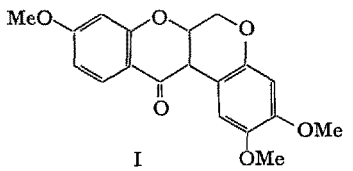
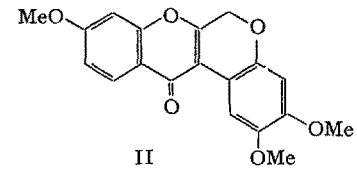
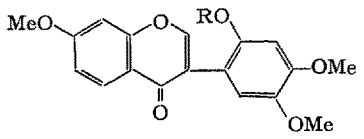


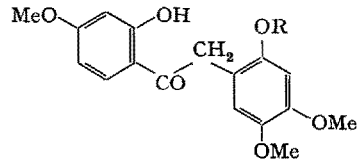
I



II



III R = Me
VI R = H
VII R = $CH_2CO_2C_2H_5$



IV R = $CH_2CO_2H$
V R = Me

acetate, the ether (IV) gave dehydromunduserone (II, m.p. 209–210°, IR 1634 $cm^{-1}$ (C = O) (Nujol), UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$); 231 (4.48), 277 (4.39), 302 (4.23). Found: C, 67.06; H, 4.69. $C_{19}H_{16}O_6$ requires: C, 67.05; H, 4.75%) (lit. [3], m.p. 210°).

The conversion of (II) into (±)-(I) has already been reported[1], this paper, therefore, completes a new synthesis of (I).

*Zusammenfassung.* Eine einfache Synthese von Dehydromunduseron aus 7,2′,4′,5′-Tetramethoxy-isoflavon wird beschrieben.

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## Enhancement of Fluorescence Emission of Acridine Orange by Nucleosides

Acridine orange (AO) is one of the basic dyes exhibiting a typical binding to polynucleotides[1–7]. When AO is bound to polynucleotides, 2 types of complexes, Complex I and Complex II, are formed, depending on the concentration ratio of polynucleotide to dye. The formation of Complex I at low concentration ratio of polynucleotide to dye brings about a new absorption band at the shorter wave-length region than the absorption peak of AO monomer and the quenching of AO fluorescence. On the other hand, the formation of Complex II at high concentration ratio of polynucleotide to dye yields a red shift of absorption band of AO and the enhancement of AO fluorescence. The former complex is considered to be formed by the metachromatical binding of AO to the phosphates of polynucleotide and the latter by the intercalation of AO between base pairs.

The present article deals with the absorption and fluorescence characteristics of AO in the presence of various nucleosides to find the elementary information about the interaction between AO and nucleic acid bases. In the aqueous solution, AO has a strong tendency to form a non-fluorescent dimer in which the transition moments of monomers are parallel to each other and perpendicular to the direction of a line connecting their centres of gravity[8,9]. Therefore, the absorption spectrum of aqueous AO solution generally consists of not only the monomer $0 \rightarrow 0$ absorption band (at 492 nm) but the dimer absorption band (at 464 nm), except for in the extremely low concentrations. The dimer absorption band overlaps upon the monomer $0 \rightarrow 1$ absorption band. The absorption feature is shown in Figure 1, curve (a), where the concentration and temperature are respectively $2.10^{-5}M$ and 6 °C.

On the addition of high concentration of adenosine, the absorption spectrum undergoes pronounced changes, as shown in Figure 1, curve (1). The dimer dissociates into monomers and the monomer absorption band shifts to longer wave-lengths by about 5nm. This absorption behaviour is quite similar to that of AO when the high concentration of DNA is added to the aqueous AO solution. The absorption spectrum has a band maximum at about 502 nm at the herring sperm DNA to AO ratio of 100:1 (Complex II), as shown in Figure 1, curve (1′). Since the fluorescence-excitation spectrum coincides exactly with the absorption spectrum, curve (1′) is known to be attributable to the AO monomer complexing with DNA. The dimer originally present dissociates completely into monomers by the addition of large excess of DNA. When AO solutions in the absence and presence of adenosine or DNA are warmed up to 70 °C, the absorption spectrum changes to the free monomer band in all cases. This is shown in Figure 1, curves (b), (2) and (2′). The broadness of curve (2′) indicates that, in the case of Complex II, some fraction of AO still remains bound as Complex II even at this temperature.

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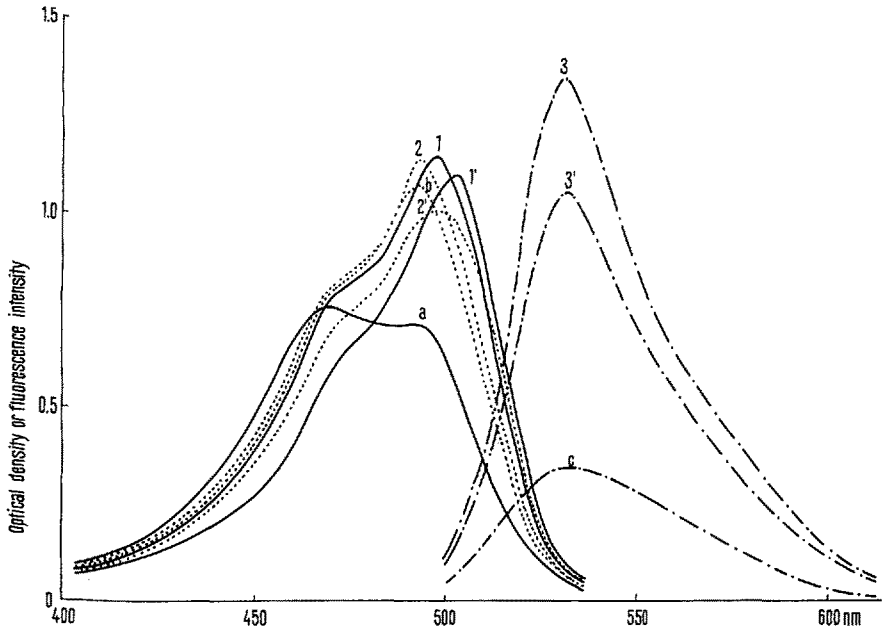


Fig. 1. Absorption and fluorescence spectra of acridine orange (AO) in the absence and presence of adenosine or DNA. Concentration of AO, $2 \cdot 10^{-5} M$; pH 7.0; (a), (b) and (c), absorption spectra at 6° and 70°, and fluorescence spectrum at 6°, respectively, in the free AO solution; (1), (2) and (3), absorption spectra at 6° and 70°, and fluorescence spectrum at 6°, respectively, in the presence of adenosine ($1.8 \cdot 10^{-2} M$); (1'), (2') and (3'), absorption spectra at 6° and 70°, and fluorescence spectrum at 6°, respectively, in the presence of herring sperm DNA ($2 \cdot 10^{-3} M$ in nucleotides); light for fluorescence excitation, monochromatic light at 492 nm.

The fluorescence spectra excited by the monochromatic light at 492 nm are shown in Figure 1, curves (c), (3) and (3'). There is no distinct difference among these fluorescence spectra, but the fluorescence intensity is highly enhanced by the presence of adenosine or DNA. The ratio of fluorescence quantum yield ($\eta$) in the presence of adenosine ($1.8 \cdot 10^{-2} M$) or DNA ($2 \cdot 10^{-3} M$ in nucleotides) to that ($\eta_0$) for free AO at the infinite dilution is calculated to be about 2. The large value of $\eta/\eta_0$ and the absorption behaviours mentioned above tell us that adenosine or the base residue in DNA interacts closely with AO monomer by complex formation, conceivably through VAN DER WAALS interaction. However, $k$ values[10] of the highest filled and lowest empty molecular orbitals of AO and adenine ($k = +0.657$, $-0.278$ for AO and $k = +0.486$, $-0.865$ for adenine) do not necessarily deny a possibility of the charge-transfer complex formation between them (charge transfer from adenine to AO). Therefore, it may be valid to consider that some degree of charge transfer interaction is involved in complexing of AO and nucleoside. The more detailed investigation is necessary for clarifying this point.

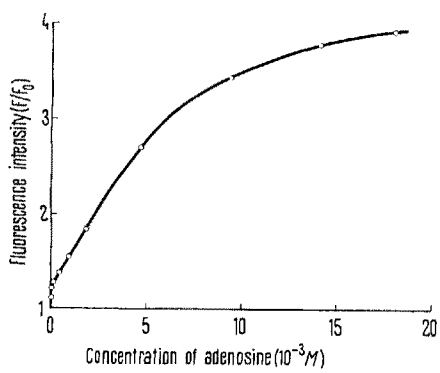


Fig. 2. Fluorescence intensity of acridine orange as a function of concentration of adenosine. Concentration of AO, $2 \cdot 10^{-5} M$; pH 7.0; temperature, 6°; exciting light, monochromatic light at 492 nm; $F_0$ and F, fluorescence intensities in the absence and presence of adenosine.

The change of fluorescence intensity is shown in Figure 2 as a function of the concentration ($C$) of adenosine. The curve consists of 3 regions: an initial very rapid rise ($C < 1 \cdot 10^{-5} M$), a subsequent more gentle rise ($1 \cdot 10^{-5} M < C < 5 \cdot 10^{-3} M$) and a gradual increase up to a saturation value ($5 \cdot 10^{-3} M < C$). These might be explained by the assumption that the presence of small amounts of nucleosides accelerate the dissociation of AO dimers by the formation of molecular complex but, at the high concentration of nucleosides, nucleoside molecules themselves aggregate with each other[11], leading to the decrease from the apparent concentration of nucleoside monomers effective for complexing with AO monomer. The other nucleosides (ribonucleosides and deoxyribonucleosides) are also effective for the enhancement of AO fluorescence emission, but adenosine and deoxyadenosine are the most effective of all: $\eta/\eta_0$ for adenosine and deoxyadenosine are, roughly speaking, about 1.3 times as large as those for the other nucleosides. This is probably due to the difference of conjugate double bond structures of nucleosides. The ability of the formation of molecular complex between AO and various nucleosides seems to be helpful for understanding the Complex II formation in DNA, RNA and the other polynucleotides.

The further details will be published elsewhere.

*Zusammenfassung.* Zusatz von Nucleosid vergrössert die Fluoreszenzintensität des Acridinorange in wässriger Lösung stark. Absorptions- und Fluoreszenz-Verhalten des Acridinorange weist auf die Bildung eines Molekülkomplexes zwischen Acridinorange und Nucleosid hin.

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[10] A. SZENT-GYÖRGYI, *Introduction to a submolecular biology* (Academic Press, New York and London 1960) p. 38. The + sign of $k$ denotes the highest filled molecular orbital and the − sign of $k$ the lowest empty one.

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