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THE SYNTHESIS AND BIOLOGICAL EVALUATION OF HYPERICIN ANALOGS

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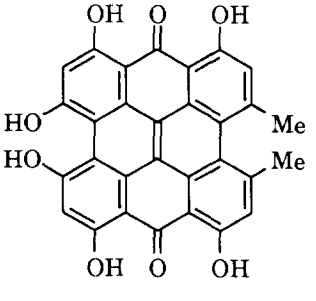
Abstract. Although the peri-hydroxyl groups in hypericin are essential for retroviral inhibitory activity, the remaining hydroxyl groups can be alkylated without loss of activity.

The advent of the human immunodeficiency virus (HIV), the virus which leads to the onset of AIDS, has spawned intense interest in retroviral research. Individuals infected with HIV become immune-compromised and are susceptible to opportunistic infections such as cryptosporidiosis and *Toxoplasma gondii*. The Center for Disease Control estimates that more than 20 million people are infected by HIV.

Recent research has led to a better understanding of retroviruses and the chemical agents that disrupt their function. Disruption at the viral replication stage is perhaps the most common mode of viral inactivation. Antiviral agents such as AZT and DDI exert their inhibitory effect by interfering with viral replication. Both AZT and DDI function as chain terminators. The eventual emergence of drug-resistant viral variants likely contributes to the fact that these therapies may delay, but do not completely block, the progression to clinical disease in HIV infected individuals.

Hypericin (**1**) is a naturally-occurring polycyclic quinone which exhibits in vitro inhibitory activity against several enveloped viruses. Hypericin is currently being used in clinical trials for HIV. Hypericin has a long history of use as a folk medicine for treating ailments as wide ranging as bed wetting, headache, rheumatism, and depression.[1] This compound is a member of a growing class of hydroxy quinones which exhibit photodynamic behavior. Related natural products such as stentorin (**2**), hypocrellin A and cercosporin



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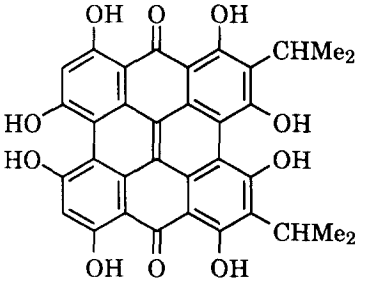
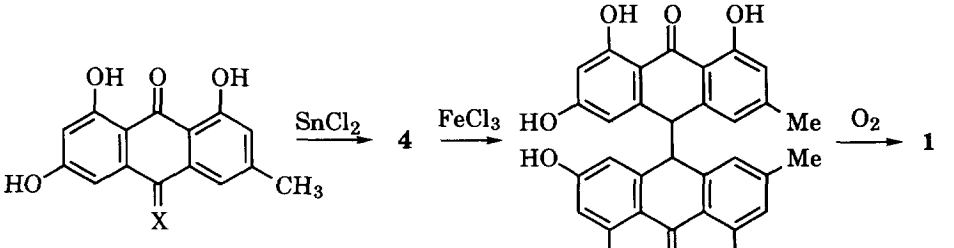


2

are members of this growing class.[2] Certain porphyrins, chlorins and phthalocyanines also exhibit photodynamic behavior. There is a rapidly-expanding literature on the use of photodynamic compounds to

inactivate tumor cells and virus-infected cells (photodynamic therapy). The literature on the photodynamic activity of hypericin has been collated in an excellent review by Song.[3]

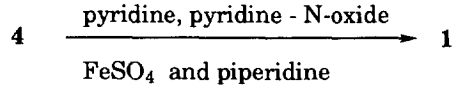
Steglich and Rodewald independently demonstrated that treatment of emodin with dilute aqueous base at 100 °C for ten days led to the production of hypericin in 1% yield.[4] Banks, Cameron and Raverty also reported a synthesis of hypericin from emodin.[5] Their synthesis involved the reduction of emodin (**3**) to its anthrone (**4**), coupling the anthrone in the presence of ferric chloride to form a bianthrone (**5**) and oxidation of the bianthrone in ammonia with oxygen. Although the yield on a millimole scale is reported to be good, this sequence appears to be difficult to scale up to generate gram quantities of hypericin. Another synthesis of hypericin from emodin



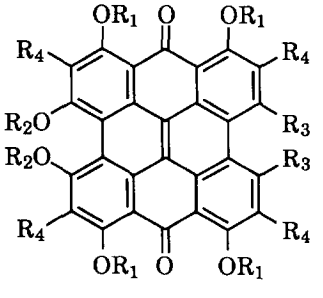
3: X=O
4: X=H, H

5

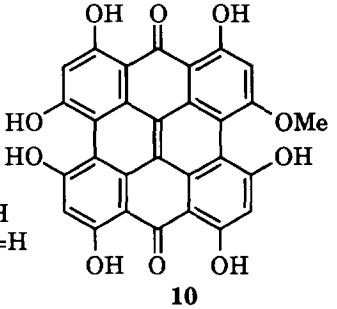
has been reported in a patent in 1992.[6] This preparation is depicted below. Falk has reported a clever and direct synthesis of hypericin using organometallic chemistry.[7]



In designing hypericin conjugates for antiviral therapy, it is important to know which functional groups on hypericin are essential for potent antiviral activity. To address this question, we synthesized analogs **6**-**10**. We had previously reported that hydroxylated anthraquinones, hydroxylated perylenequinones and polycyclic



6: R_1=R_2=Me, R_3=OMe, R_4=H
7: R_1=H, R_2=Me, R_3=OH, R_4=H
8: R_1=R_2=H, R_3=OH, R_4=H
9: R_1=R_2=H, R_3=OH, R_4=Me

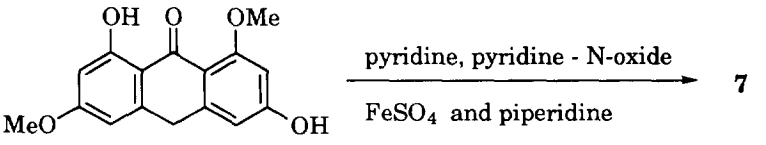


10

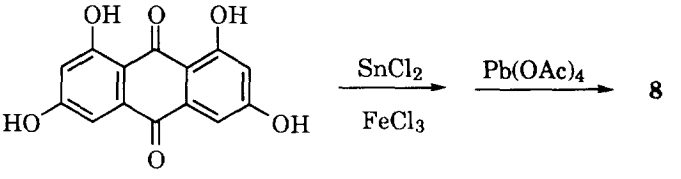
quinones which resembled hypericin did not exhibit significant antiviral activity.[8] Analogs **6** - **10** assess the relative importance of hydroxyl groups on the phenanthro-perylene ring. Analog **6** was prepared from the

anthrone of 1,3,6,8-tetramethoxyanthraquinone. On the basis of ultrafast spectroscopic studies of hypericin and related hydroxy quinones, Petrich has proposed that the hydroxyl groups peri to the quinone carbonyl groups contribute to the antiviral activity in the presence of oxygen and are essential to the antiviral activity in the absence of oxygen.[9] The testing of analog **6** will probe this hypothesis.

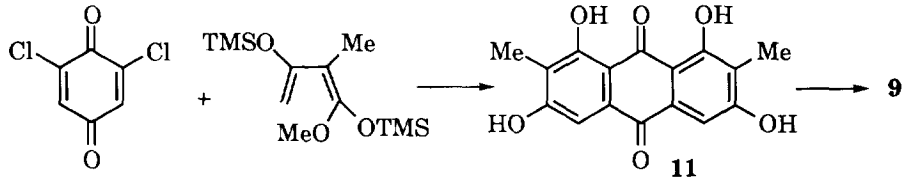
Analog **7** was synthesized as shown below. Analog **10** was produced in low yield as a by-product of the synthesis of **7**.



Analog **8** was readily prepared from 1,3,6,8-tetrahydroxy anthraquinone by coupling of the corresponding anthrone followed by oxidative cyclization. We have found that lead tetraacetate is a superior oxidant in this case.

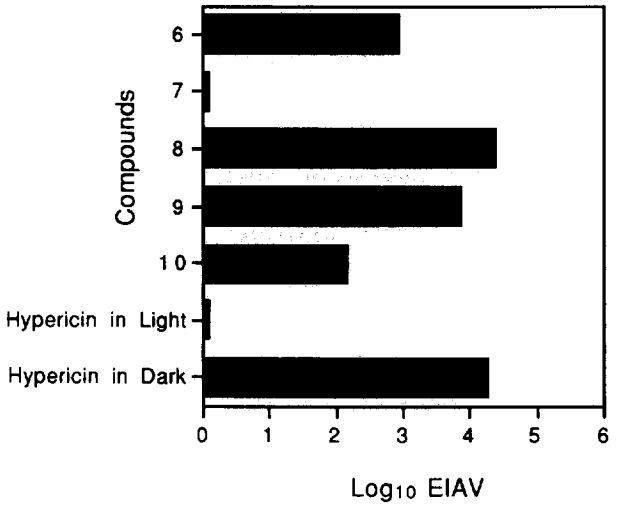


Anthrone **9** was efficicently prepared from 1,3,6,8-tetrahydroxy-2,7-dimethyl anthraquinone (**11**), which in turn was synthesized from 2.6-dichlorobenzoquinone by a double Diels-Alder reaction. Since anthraquinone **11** has only one site for radical coupling, we tried to make **9** using the conditions developed by Steglich. Unfortunately, after **11** was treated with base for 7 days at 100 °C, only starting material was recovered. However, subjecting **11** to reduction ($SnCl_2$), coupling ($FeCl_3$) and oxidation ($PbOAc_4$) furnished



analog **9** in 68% yield.

Analogs **6**-**10** were tested for anti-retroviral activity in vitro using the equine infectious anemia virus (EIAV).[10] EIAV is a retrovirus that is antigenically similar to HIV. Analogs **8**[11] and **9** did not exhibit any antiviral activity, as evidenced by the lack of a decrease in the number of focus-forming units (FFU). Analogs **7** showed similar activity to hypericin. Our results indicate that **6** and **10** possess activity which is considerably



reduced with respect to that of hypericin. One explanation for the dramatically reduced activity compared to hypericin is that the two additional hydroxyl groups render these molecules more soluble in water and do not drive the molecules into the viral membrane. Hypericin is insoluble in water at pH 4-8.

The antiviral activity of hypericin and its analogs is likely the net result of an interplay between the redox potential and the molecule's ability to partition between the cell membrane and water.[12] The construction of conjugates which retain the potent antiviral activity of hypericin seems limited to functionalization of the hydroxyl groups not involved in hydrogen bonding to the carbonyl group.

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