Tetrahedron Vol. 45, No. 8, pp. 2263 to 2276, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00
Pergamon Press plc

Reaction Models of the Oxidative Rearrangement of Averufin to 1'-Hydroxyversicolorone: The First Step in Dihydrobisfuran Formation in Aflatoxin Biosynthesis

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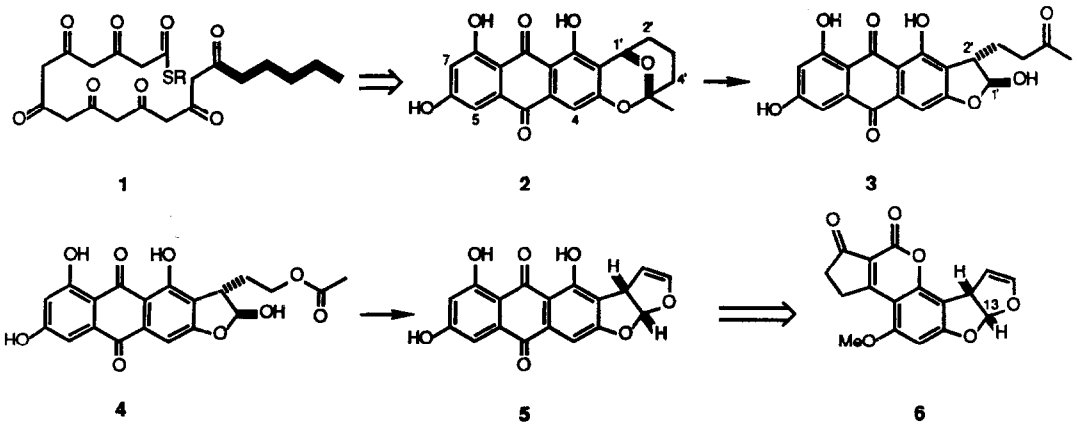
(*Received in South Africa* 11 *August* 1988)

Abstract—The formation of the dihydrobisfuran ring system uniquely representative of the aflatoxins among polyketide natural products is achieved in three net oxidative steps relating averufin (2) to versicolorin A (5). The first of these oxidative rearrangements carries the former to 1'-hydroxyversicolorone (3). Labeling experiments have placed several limitations on the mechanisms of reactions that can account for this transformation. Two viable possibilities involve radical or cationic intermediates whose fundamental reactivity toward rearrangement has been investigated in model reactions based on derivatives of 6,8-di-*O*-methylaverufin (20, X=H).

The formation of the dihydrobisfuran ring system characteristic of aflatoxin B_1 (6) poses a set of mechanistically intriguing problems in enzymatic rearrangement chemistry. These processes are oxidative and relate the key intermediate averufin (2),[1] an anthraquinone of clear polyketide origin, to versicolorin A (5),[2] the first dihydrobisfuran-containing structure of the pathway (see Scheme I). Elevation of the side chain oxidation state from 2 to 5 (three net oxidations) has been mapped through 1'-hydroxyversicolorone (3), the product of a chain-branching step, and versiconal acetate (4), the result of an apparent biochemical Baeyer-Villiger reaction. 1'-Hydroxyversicolorone has been isolated from a new mutant of *Aspergillus parasiticus (hvn-1)* [3]and shown to give efficient, intact incorporations of label into aflatoxin B_1[4] while earlier work has demonstrated the probable intermediacy of versiconal acetate.[2] In this paper we focus in some detail on the chemical nature of the first of these oxidative steps, the conversion of averufin (2) to hydroxyversicolorone (3).



Scheme I

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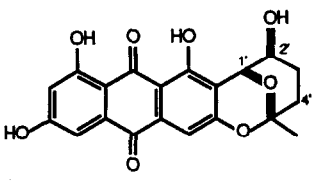
Our initial thoughts about this chain-branching step were that nidurufin (**7**), a known metabolite of *A. nidulans*,[5] presented an ideal *anti*-periplanar[6] orientation of migrating and leaving groups to facilitate its rearrangement (presumably the hydroxyl would have to be activated in some way, *e.g.* phosphorylation). However, contrary to our expectations, neither labeled nidurufin nor its 2'-epimer, pseudonidurufin, gave any detectable incorporation into aflatoxin B_1.[7] Consideration of this disappointing result led to a more subtle test of the presumed stereoelectronic advantages[6,7] inherent to the above mechanistic proposal. If the rearrangement were to proceed through the closed form of the bicyclic ketal side chain, the 1'-oxygen (•)/5'-carbon (■) bond (*cf.* **9**, R=H; Scheme II) should remain intact to label the acetate of versiconal acetate as shown in **11**. A range of alternative mechanisms could be imagined but if the ketal of **2/9** were opened, these labeled centers would no longer be connected. ^{18}O-Induced isotopic shifts were observed[7] at C-5' in **11**, clearly supporting the first of these two mechanistic possibilities. Moreover, recent stereochemical studies examining the fate of [2-$^2H_3$,^{13}C]acetate in averufin have shown that deuterium label occupies the *equatorial* locus at both C-2' and C-4'.[8] As both of these deuteria survive the oxidative steps that lead to formation of the dihydrobisfuran,[9] it must, therefore, be true that the 2'-axial hydrogen (H°) is specifically lost during the oxidative rearrangement of averufin (**8**) to hydroxyversicolorone, in keeping with the essential determinants of the stereoelectronic argument above.



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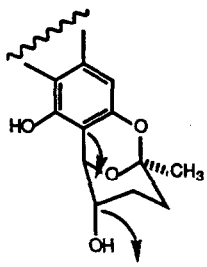

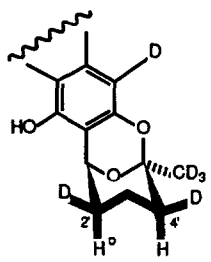

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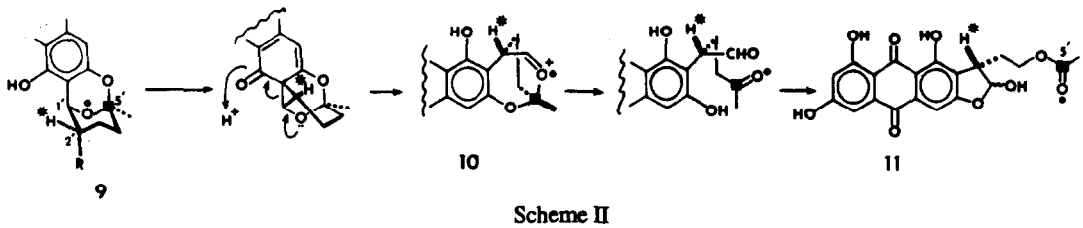
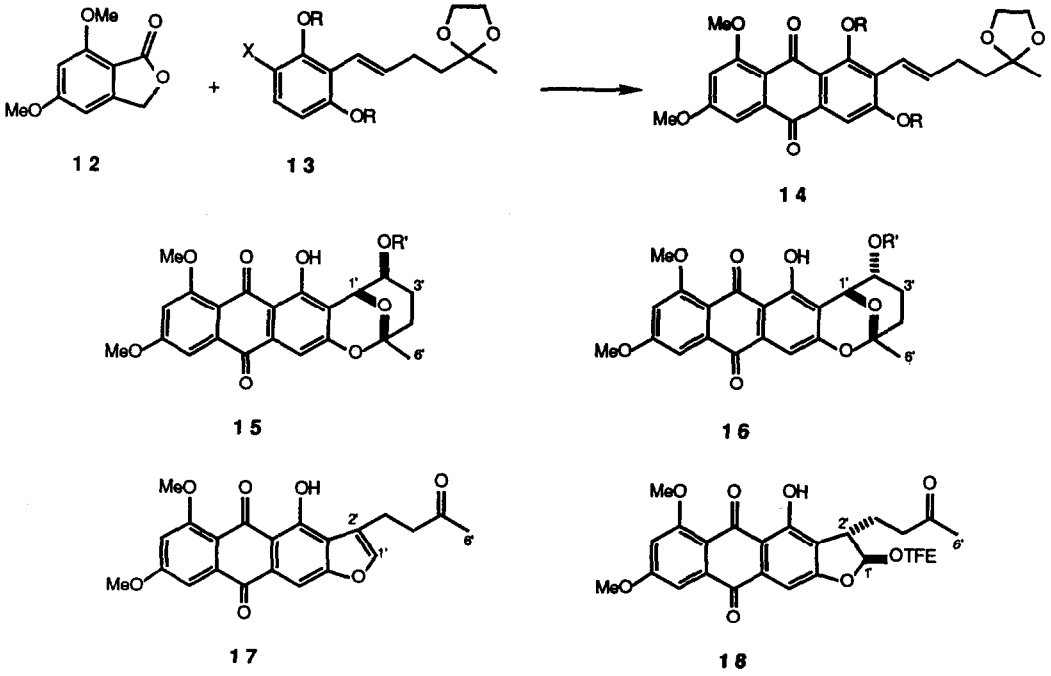
The rearrangement of averufin (**2**) to hydroxyversicolorone (**3**) takes place with complete retention of the 1'-hydrogen (which appears at C-13 in **6**)[1] and stereospecific loss of the *exo*-2'-hydrogen;[8] that is, the hydrogen *trans*-diaxial to the migrating anthraquinone nucleus is lost in an overall process that is clearly oxidative. Yet this pinacol-like rearrangement[6] *in vivo* fails to utilize the obvious oxidized intermediate, nidurufin (**7**). As has been discussed elsewhere,[7,10] one is led by these findings to the view that the rearrangement itself occurs through a reactive radical or cation intermediate generated from **2** in the course of an otherwise normal hydroxylase reaction. Activation, therefore, of the nidurufin hydroxyl for ionization or displacement is avoided. Model studies[11] carried out to explore the chemical nature of a reaction path involving ionization/rearrangement suggest that there would be significant kinetic barriers to be overcome by the enzyme responsible for such a process were nidurufin to be involved in the biosynthesis.

The model systems examined were the mesylates of 6,8-di-*O*-methylnidurufin (**15**, R'=Ms) and 6,8-di-*O*-methylpseudonidurufin (**16**, R'=Ms) under solvolytic conditions.[11] These substrates were prepared by adapting methods developed in an earlier synthesis of averufin.[12] The anion of 5,7-dimethoxyphthalide (**12**)[12,13] was reacted with the benzyne[12] derived from dehydrobromination of the aryl bromide **13** (X=Br) to give, after air oxidation and silica gel chromatography, the fully protected anthraquinone **14**. Conversion of **14** to its epoxide[14]



Scheme II

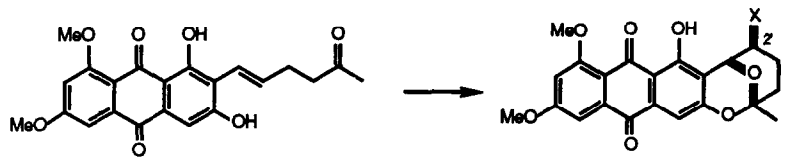
and acid-catalyzed rearrangement gave a separable mixture of 6,8-di-*O*-methylnidurufin (**15**, R'=H) and 6,8-di-*O*-methylpseudonidurufin (**16**, R'=H) in overall yields of 28% and 11%, respectively. In 2,2,2-trifluoroethanol (TFE) at 80 °C the nidurufin derivative **15** (R'=Ms) underwent smooth but slow rearrangement showing clean first-order kinetics ($t_{1/2}$= 3.7±0.1 h) to a 1:4 mixture of the fused furan **17** and the mixed acetal **18**. In contrast the corresponding pseudonidurufin derivative **16** (R'=Ms) remained unchanged on prolonged heating apart from gradual decomposition to dark polymeric material.[11] With the hope that added base might enhance the rate of rearrangement (by deprotonation of the anthraquinone 1-OH), the solvolysis reactions were monitored in TFE (pK_a *ca.* 12)[15] containing 2% triethylamine. While the *endo*-mesylate **16** (R'=Ms) remained unreactive, the *exo*-mesylate **15** (R'=Ms), as expected, rearranged notably faster ($t_{1/2}$ *ca.* 100 min., 45 °C), but not to the furanoid products **17** and **18** as before, but to an 8.8:1 mixture of the TFE ethers **15** (R'=CH_2CF_3) and **16** (R'=CH_2CF_3). The principal further observations made in these studies have been published and will not be repeated here.[11] We provide, however, full details in the Experimental section of these earlier studies: the preparation of **15** and **16**, characterization of their solvolysis products under two sets of reaction conditions noted above and a description of the kinetic analyses.



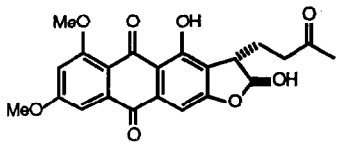
Several conclusions could be drawn at this point about the fundamental reactivity of nidurufin and pseudonidurufin toward rearrangement in a cationic regime. First, the markedly dissimilar behavior of the *endo*- and *exo*-mesylates is consistent with a large body of literature[16] in which the π-system of the migrating group can interact with the antibonding orbital of a departing group oriented *anti*-periplanar in a transition state that leads to branched chain products, as in the case of nidurufin. In pseudonidurufin these orbital relationships are orthogonal and rearrangement fails to occur. Second, even for the case of 6,8-di-*O*-methylnidurufin mesylate (**15**, R'=Ms), the rate of rearrangement is quite slow. The 1'-oxygen, which might be thought to stabilize a developing positive charge at C-1' in the transition state, in fact acts in precedented fashion to oppose charge development at this center through strong inductive electron withdrawl.[17] Rearrangement is further retarded by the fact that the anthraquinone nucleus as a migrating group is fundamentally electron-poor.[18] While it might be argued that an enzyme could have evolved to overcome the kinetic barriers inherent to rearrangement of this substrate, the fact remains that nidurufin is not involved in the biosynthesis of aflatoxin.

Biological oxidations at unactivated carbon sites are currently held (at least for the comparatively well-studied cytochromes P-450) to take place by way of carbon-centered radicals.[19] Electron transfer could then give a carbonium ion. For averufin (**2**/**9**, R=H), as discussed earlier, one or the other of these species would be required to undergo rearrangement (Scheme II) to give hydroxyversicolorone (**3**) where intermediate **10** as a heteroatom-stabilized radical or oxonium ion could be visualized to consumate the hydroxylation/hydration reaction. 1,2-Aryl migrations have been studied in radical reactions.[20] Both electron-donating and electron-withdrawing substituents accelerate the rates of these rearrangements but more as a result of delocalization of the unpaired electron than through stabilization of a polar transition state. For averufin such a process in the radical regime would have the appealing features of using the migrating anthraquinone nucleus to delocalize the unpaired electron (quinones are widely associated with single-electron processes in Nature) and the initially-formed 2'-radical would, as a result of rearrangement, become stabilized by an adjacent heteroatom in **10**.

To explore the *in vitro* feasibility of a radical rearrangement process, the fully protected anthraquinone **14** was treated with 1*N* hydrochloric acid in THF to give 1,3-dihydroxy-6,8-dimethoxy-2-(5-oxo-1-hexenyl)anthraquinone (**19**) and a small amount of *exo*-2'-chloro-6,8-di-*O*-methylaverufin (**20**, X=Cl). The corresponding *exo*-bromo (**20**, X=Br) and iodo-derivatives (**20**, X=I) could be readily obtained by treating **19** in aqueous THF with N-bromosuccinimide or iodine/silver oxide,[4,21] respectively. All attempts, however, to observe rearrangement of the 2'-radical generated from **20** (X=Br or I) failed under a variety of reaction conditions: heating to reflux in benzene or toluene in the presence of AIBN and tri-*n*-butylstannane[22] or tri-*n*-butylgermane[23] as well as protocols using high dilution or slow addition by syringe pump. Only the reduction product 6,8-di-*O*-methyl averufin (**20**, X=H; 15-65% isolated yields) was detected together with unreacted starting material. In a few instances, despite conduct of the reactions under an inert atamosphere, trace amounts of 6,8-di-*O*-methylnidurufin (**20**, X=OH) were obtained, owing presumably to trapping of the 2'-radical by molecular oxygen to give the hydroperoxy radical **20** (X=O-O•), which was further reduced to **20** (X=OH). Brief trials to decrease the rate of reduction of the 2'-radical were made using the di-*tert*-butyl peroxide/trimethylsilane method.[24] Only the starting iodide **20** (X=I) and its simple reduction product **20** (X=H) were obtained. Other experimental protocols to prolong the lifetime of the 2'-radical to allow time for its rearrangement were similarly unsuccessful.[25]



19 20



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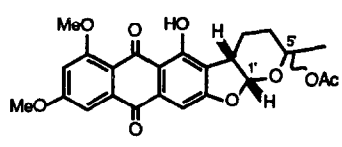

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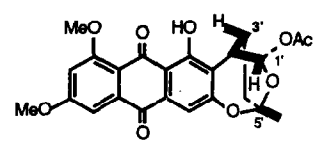

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The failure of the *exo*-iodide **20** (X=I) to give reduced rearrangement products under radical generating conditions led us to examine again this process in the cationic regime with the hope that a *quasi* biomimetic synthesis of 1'-hydroxyversicolorone (**3**) might be achieved. Success was indeed met and an unexpected observation was made in the course of these experiments that gave particular support to the mechanism depicted in Scheme II. Reaction of the *exo*-iodide **20** (X=I) in several organic solvents, notably dioxane, with silver trifluoroacetate gave a single product when monitored by thin layer chromatography, 6,8-di-*O*-methyl-1'-hydroxyversicolorone (**21**). Unoptimized yields of isolated material were 70% after 20 hours of reaction at room temperature with some starting material yet remaining. However, reaction in the presence of silver acetate gave **21** in only small amounts together with 75-85% yields of a new product. Addition of 1-1.5 equivalents of water accelerated the overall rate of reaction and greatly reduced the amount of this new product. Hydrolysis of this product in THF/1*N* hydrochloric acid gave **21**. ^{1}H-NMR and mass spectral data obtained for this material showed that the iodine present in **20** (X=I) had been replaced in the new product by acetoxy, but an acetal methyl (δ 1.59) and the anthraquinone nucleus had clearly been retained in what appeared to be a single diastereomer. Chemical shift comparisons and homonuclear decoupling experiments led to two possible structures, **22** and **23**. Assignment of the ^{13}C-NMR spectrum required that C-1' and C-5' in **22** have chemical shifts of δ 94.3 and 103.1, respectively, while in **23** these assignments would have to be reversed. Chemical shift comparisons[3,6] supported structure **23**, but definitive proof came on reaction of **20** (X=I) with silver [1-^{13}C]acetate to give **23** in which, *inter alia*, H-1' was split not only by w-coupling to H-3' (^4J = 0.8 Hz), but also by three-bond coupling to the now labeled acetate carbonyl ($^3J_{CH}$ = 2.8 Hz). This was a pleasing result since the mechanism proposed in Scheme II involves rearrangement through the closed form of the ketal side chain of averufin in which migration of the anthraquinone nucleus occurs across one face. Acetate ion being more nucleophilic than trifluoroacetate traps the bridged intermediate from the opposite face to give **23** as a single diastereomer in a classic manifestation of neighboring group participation.

From the perspective of *in vitro* model chemistry, the C-2 radical generated from *exo*-iodide **20** (X=I) failed to yield any detectable reduced rearrangement products under a variey of reaction conditions. It may be that this bond reorganization process is possible but that it simply is unable to compete with reduction of the initially-formed radical to give 6,8-di-*O*-methylaverufin **20** (X=H) under the experimental conditions tried. On the other hand, while the mesylate **15** (R'=Ms) shows rates of solvolysis in TFE that indicate significant kinetic barriers to

ionization/rearrangement,[11] it, like the *exo*-iodide **20** (X=I) on reaction with silver salts, does give furanoid products in good yield. Incorporation experiments have demonstrated that nidurufin (**7**) is not involved in the biosynthesis of aflatoxin B_1 (**6**).[7] Further labeling experiments have imposed severe constraints on the oxidative rearrangement of averufin (**2**) to hydroxyversicolorone (**3**). As shown in Scheme II, [1'-^{18}O,5'-^{13}C]averufin (**9**, R=H) gives versiconal acetate labeled as in **11**,[7] and it has been established that the *exo*-2'-hydrogen (H°) in averufin (**8**) is specifically lost in the formation of hydroxyversicolorone.[8] All of these data, from both chemical and biosynthetic experiments, support a cationic reaction path as proposed in Scheme II involving the intermediacy of **10**, whose trapping product **23** was observed in a model reaction. However, to extrapolate these findings to the catalytic mechanism of an enzyme that has not been isolated or directly studied, of course, has well-recognized limitations. Nonetheless, the chemical constraints imposed by the fundamental reactivity of the substrate cannot be ignored and they define more sharply important questions about the enzymatic oxidative rearrangement process.

EXPERIMENTAL

Melting points were determined in open capillaries using a Thomas-Hoover apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer model 599B or Series 1600 FTIR spectrometer. NMR spectra were obtained using a Varian XL-400 spectrometer unless otherwise noted; chemical shifts (δ) are reported downfield of tetramethylsilane as internal standard; cm indicates a complex multiplet, sym m represents a symmetrical multiplet, other abbreviations are those conventionally used. Mass spectra were obtained through the services of mass spectrometry facilities at MIT, Pennsylvania State University, University of Minnesota, the Middle Atlantic Mass Spectrometry Laboratory at the Johns Hopkins Medical School (Kratos MS-50) and in the Department of Chemistry, Johns Hopkins University (VG Micromass 70-S). Elemental combustion analyses were carried out by Galbraith Laboratories, Inc. (Knoxville, TN). HPLC was performed with a Perkin-Elmer Series 4 liquid chromatograph using a Perkin-Elmer LC 85B variable wavelength UV-visible detector. Silica gel chromatography, solvent purification, standardization of lithium bases and composition of baths used for low-temperature reactions have been described elsewhere.[1] Reactions were carried out in oven-dried glassware under an atmosphere of dry nitrogen unless otherwise stated.

Aryl Bromide 13 (X=Br, *trans*-olefin). A 50 mL round-bottomed flask containing the *trans*-olefin **13** (X=H; 810 mg, 2.39 mmol) was equipped with a magnetic stirring bar, a rubber septum and an argon inlet (needle) and was purged with argon for 5 min. Dry ether (12 mL) was added and the mixture was stirred under argon as *n*-BuLi in hexane (1.8 mL. 2.87 mmol) was added in one portion to produce a yellow solution, with a precipitate forming 5 min later. At 1 h, the mixture was sonicated for *ca.* 1 min to dislodge the precipitate clinging to the walls of the flask. This procedure was repeated at 2.3 h, after which a solution of cyanogen bromide in dry ether (1.7 mL, 3.65 mmol) was added in one portion to produce a dark brown mixture. This was sonicated for *ca.* 2 min and then stirring was continued for 30 min. Water was added and the mixture was extracted three times with ether. The ether extracts were pooled, washed with water, brine and dried over anhydrous potassium carbonate. Filtration and concentration *in vacuo* provided a brown oil, which was chromatographed on a column of silica gel (100 g, 50 × 2.4 cm, ether:pentane, 1:1) to provide bromide **13** (X=Br; 809 mg, contained *ca.* 3% by weight of starting material, 785 mg, 78.6%) as a colorless oil. In order to obtain pure compound for analysis, a portion (100 mg) of this product mixture was chromatographed on a column of silica gel (100 g, 50 × 2.4 cm, pentane:ether, 2:1), providing pure bromide **13** (X=Br; 74 mg; the remainder of the bromide contained unbrominated hydrocarbon) as a colorless oil. IR ($CHCl_3$): 2980, 2950, 2890, 2830, 1580, 1565, 1460, 1390, 1380, 1260, 1160, 1035, 975, 950, 925, 865 cm^{-1}; ^{1}H NMR (80 MHz, Varian CFT-20; $CDCl_3$): δ 7.31 (d, J = 8.9 Hz, 1H, H-4) 6.80 (d, J = 8.9 Hz, 1H, H-5), 6.52

(unsym t, J_{app} = 3.8, 2.6 Hz, 2H, *trans*-CH=CH), 5.15 (s, 2H, OCH_2O), 5.02 (s, 2H, OCH_2O), 3.96 (s, 4H, OCH_2CH_2O), 3.63 (s, 3H, OMe), 3.46 (s, 3H, OMe), 2.50–2.10 (cm, 2H, H-3'), 1.92–1.65 (cm, 2H, H-4'), 1.35 (s, 3H, H-6'). TLC (pentane:ether, 3:2) R_f : 0.27 [R_f of starting olefin **13** (X=H): 0.31]; (pentane:ether, 3:2) R_f 0.51 (R_f of starting olefin: 0.55). Anal. Calcd for $C_{18}H_{25}O_6Br$: C, 51.81; H, 6.04%. Found C, 51.69; H, 6.06%.

1,3-Dimethoxymethoxy-6,8-dimethoxy-2-[5-(1,3-dioxolane-2-yl)-*trans*-1-hexenyl]anthraquinone (14). A 250 mL three-necked flask was fitted with an addition funnel and charged with 10.4 mL of THF and 2,2,6,6-tetramethylpiperidine (1.36 mL, 1.14 g, 8.05 mmol). The reaction was cooled to –78 °C (dry ice/methylene chloride cold bath), after which *n*-butyllithium (5.4 mL of a 1.50 M solution in hexane, 8.05 mmol) was added dropwise. The reaction was allowed to stir at this temperature for ten min., at which time 5,7-dimethoxyphthalide (**12**; 446 mg, 2.30 mmol)[13] in 26 mL of THF and 2.5 mL of HMPA was added dropwise. The resulting yellow-orange solution was allowed to stir for 20 min., at which time the reaction mixture was warmed to –45 °C (dry ice/acetonitrile cold bath). A mixture containing 9:1 bromide **13** (X=Br)/olefin **13** (X=H) [2.05 g, 4.55 mmol of bromide **13** (X=Br)] was added dropwise to the reaction vessel in 16 mL of THF. The reaction mixture immediately turned bright red and then purple as it was allowed to stir at this temperature for another 40 min. The cold bath was removed and stirring was continued for another h, at which time the reaction was opened to the air and *immediately* quenched with 120 mL of water. The mixture was extracted with ethyl acetate (4 × 60 mL). The combined organic extracts were then washed with brine (2 × 60 mL), dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. Isolation was by short path column chromatography (130 g silica gel, ethyl acetate:pentane, 1:1) to yield tetraprotected anthraquinone **14** as a yellow foam (433 mg, 36%, mp 82.5–87.5 °C). IR ($CHCl_3$): 3005, 2940, 2850, 1665, 1600, 1570, 1465, 1430, 1385, 1380, 1325, 1300, 1260, 1160, 1130, 1065, 1035, 1000, 975, 950, 925, 915 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.69 (s, 1H, H-4), 7.33 (d, J = 2.7 Hz, 1H, H-5), 6.74 (cm, 3H, H-7 and *trans*-CH=CH), 5.36 (s, 2H, OCH_2O), 5.16 (s, 2H, OCH_2O), 3.94-4.00 (distorted q from an AA'BB' spin system, 4H, OCH_2CH_2O), 3.96 (s, 3H, OMe), 3.95 (s, 3H, OMe), 3.59, (s, 3H, OMe), 3.51 (s, 3H, OMe), 2.38-2.44 (sym m, 2H, H-3'), 1.84-1.88 (sym m, 2H, H-4'), 1.37 (s, 3H, H-6').

Owing to the comparatively ready loss of the 1-methoxymethyl group, the tetraprotected anthraquinone **14** was converted to the dihydroxyketone **19** for the purpose of analysis.

1,3-Dihydroxy-6,8-dimethoxy-2-(5-oxo-1-hexenyl)anthraquinone (19). Anthraquinone **14** (205.7 mg, 0.39 mmol) was dissolved in THF (30 mL) and 1*N* HCl (10 mL) was added. The solution was heated to a gentle reflux for 17 h. After cooling to room temperature, the solution was concentrated *in vacuo* and the mixture was extracted with ethyl acetate (2 × 30 mL) and the combined extracts were washed with brine (2 × 10 mL), dried over anhydrous magnesium sulfate and concentrated under vacuum. The residue was subjected to flash chromatography in silica gel (7 g) eluting with chloroform to give 1,3-dihydroxy-6,8-dimethoxy-2-(5-oxo-1-hexenyl)anthraquinone (**19**) as a coral red solid, 121.2 mg (78.6% yield; mp 224–225 °C, recrystallized from THF). IR (KBr): 3270 (br), 2900 (br), 1700, 1660, 1610, 1585, 1420, 1310, 1270, 1160 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.92 (s, 1H, 1-OH), 7.46 (d, J = 2.4 Hz, 1H, H-5), 7.29 (s, 1H, H-4), 6.78 (d, J = 2.4 Hz, H-7), 6.72 (s, 1H, 3-OH), 6.53 (d, J = 16.5 Hz, 1H, H-1'), 6.40 (t x d, J = 6.4, 16.5 Hz, 1H, H-2'), 4.02 (s, 3H, OMe), 3.98 (s, 3H, OMe), 2.70 (t, J = 6.8 Hz, 2H, H-4'), 2.58 (apparent q, Japp = 6.8 Hz, 2H, H-3'), 2.21 (s, 3H, H-6'); MS, *m/z* 396(M^+, 49%), 354, 353, 339, 338 (100), 337, 325; accurate mass 396.1210, calcd for $C_{22}H_{20}O_7$ 396.1209.

A small amount of *exo*-2'-chloro-6,8-di-*O*-methylaverufin (21.7 mg, 12.9% yield; mp 256–257 °C dec., recrystallized from benzene) was isolated from the silica gel chromatography. IR ($CHCl_3$): 3540 (br), 3010, 2940, 2830, 1675, 1625, 1600, 1560, 1395, 1325, 1290, 1265, 1235, 1160, 990, 890 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.62 (s, 1H, 1-OH), 7.44 (d, J = 2.4 Hz, 1H, H-5),

7.21 (s, 1H, H-4), 6.78 (d, J = 2.4 Hz, 1H, H-7), 5.33 (br s,1 H, H-1'), 4.36 (br q, J = 2.2 Hz,1H, H-2'), 4.02 (s, 3H, OMe), 3.98 (s, 3H, OMe), 2.34 (d x t, J = 5.6, 14.0 Hz, H-4'$_{ax}$), 2.07 (d x d x t, J = 3.6, 5.2, 15.2 Hz, 1H, H-3'$_{ax}$), 1.87-1.99 (apparent d x t, Japp = 5.2, 14.0 Hz, 2H, H-3'$_{eq}$, H-4'$_{eq}$), 1.65 (s, 3H, H-6'); MS *m/z* 432 (5.6%), 430 (M^+, 17.0%), 396, 395 (100%), 394, 351, 337, 325; accurate mass 430.0805, calcd for $C_{22}H_{19}O_7Cl$ 430.0819.

6,8-Di-*O*-methylnidurufin (15, R'=H) and 6,8-Di-*O*-methylpseudonidurufin (16, R'=H). A 100 mL round-bottomed flask was charged with tetraprotected anthraquinone **14** (500 mg, 0.947 mmol), 40 mL of methylene chloride, and 13.4 mL of a 0.5 *M* aqueous solution of sodium bicarbonate. 3-Chloro-peroxybenzoic acid (328 mg, 1.89 mmol) was added, and the reaction was allowed to stir for 3 h. The reaction was then quenched by adding 130 mL of ethyl acetate and washing with 5% aqueous potassium carbonate (2 × 40 mL) and brine (1 × 40 mL). The organic layer was dried over anhydrous magnesium sulfate and the solution was concentrated *in vacuo*. The yellow solid which was obtained (510 mg), the 1',2'-epoxide, could be used in the next reaction without further purification. For purposes of characterization, the product was purified by short path column chromatography (50:1 silica gel to compound, ethyl acetate:pentane, 1:1) to give a yellow foam (mp 109.5–112 °C). IR ($CHCl_3$): 2960, 2930, 2970, 2960, 1670, 1600, 1460, 1380, 1325, 1260, 1160, 1060, 915 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.70 (s, 1H, H-4), 7.32 (d, J = 2.4 Hz, 1H, H-5), 6.77 (d, J = 2.4 Hz, 1H, H-7), 5.37 (br s, 2H, OCH_2O), 5.26 (d, J_{gem} = 6.2 Hz, 1H, A of ABq, OCH_2O), 5.19 (d, J_{gem} = 6.2 Hz, 1H, B of ABq, OCH_2O), 3.96 (s, 3H, OMe) 3.95 (s, 3H, OMe), 3.95-4.00 (br s, 4H, OCH_2CH_2O), 3.94 (d, J = 2.0 Hz, 1H, H-1'), 3.61 (s, 3H, OMe), 3.53 (s, 3H, OMe), 3.39 (cm, 1H, H-2'), 1.65-1.98 (2 x cm, 4H, H-3', -4'), 1.36 (s, 3H, H-6').

This material was transferred to a 100 mL round-bottomed flask and 100 mL of dimethylsulfoxide were added. The solution was cooled with an ice bath until it just began to solidify. The ice bath was removed and 17 mL of 0.8 *N* aqueous sulfuric acid were added. The reaction mixture was then stirred for 4.5 h, at which time 150 mL of water were added. The reaction mixture was worked up in the usual way with ethyl acetate and water and, after drying the organic extract over anhydrous magnesium sulfate, removal of the solvent *in vacuo* gave a glassy orange solid. This was transferred to a 250 mL round-bottom flask, to which 65 mL of anhydrous methanol and 46 mL of glacial acetic acid were added. The reaction was then heated to reflux for 20 h. At this time the reaction was quenched with the addition of 150 mL of water and the mixture was extracted with ethyl acetate (3 × 60 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate (4 × 50 mL) and brine (2 × 60 mL), followed by drying over anhydrous magnesium sulfate and concentrating *in vacuo*. The products were isolated by flash chromatography (150 g, 230–400 mesh silica, methylene chloride:ethyl acetate, 3:2). Isolated was (±)-6,8-di-*O*-methylnidurufin (R_f = 0.48, 109 mg, 28%, mp 210.5–211.5 °C, lit.[26] mp 211–213 °C.). IR (KBr): 3480, 2930, 1655, 1620, 1585, 1555, 1435, 1380, 1345, 1325, 1290, 1240, 1210, 1160, 1050, 990, 965, 845 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.59 (s, 1H, OH), 7.47 (d, J = 2.5 Hz, 1H, H-5), 7.22 (s, 1H, H-4), 6.80 (d, J = 2.5 Hz, 1H, H-7), 5.26 (d, J = 1.7 Hz, 1H, H-1'), 4.08 (m, 1H, H-2'), 4.03 (s, 3H, OMe), 3.99 (s, 3H, OMe) 1.75–2.2 (3 x cm, 4H, H-3', -4'), 1.64 (s, 3H, H-6'). MS, *m/z* 412 (M^+, 43%), 394, 379, 369, 351, 314 (100%), 296, 99.

Also isolated was (±)-6,8-*O*-methylpseudonidurfin (43 mg, 11%, R_f = 0.43, mp 213–215 °C, recrystallized methylene chloride/ethyl acetate). IR (KBr): 3430, 2920, 1655, 1590, 1390, 1325, 1290, 1265, 1245, 1180, 1160, 980, 925, 875, 840, 755 cm^{-1}; ^1H NMR ($CDCl_3$): δ 14.02 (s, 1H, OH), 7.46 (d, J = 2.5 Hz, 1H, H-5), 7.25 (s, 1H, H-4), 6.79 (d, J = 2.5 Hz, 1H, H-7), 5.41 (d, J = 4.4 Hz, 1H, H-1'), 4.25-4.32 (cm, 1H, H-2'), 4.02 (s, 3H, OMe), 3.99 (s, 3H, OMe), 1.8–2.2 (2 x cm, 4H, H-3', -4'), 1.59 (s, 3H, H-6'); MS, m/z 412 (M^+, 88%), 394, 369, 351, 313 (100%), 296, 285, 270, 255, 99. Anal. calcd for $C_{22}H_{20}O_8$: C, 64.07%; H, 4.89%. Found: C, 63.72%; H, 5.06%.

2'-(*O*-Methanesulfonyl)-6,8-di-*O*-methylpseudonidurufin (16, R'=H). A 10 mL round-bottomed flask was charged with 6,8-di-*O*-methylpseudonidurufin (5.6 mg, 0.0136 mmol), 3.5 mL of methylene chloride, and triethylamine (6.6 μL, 4.8 mg, 0.0476

mmol). The reaction mixture was cooled to 0 °C with an ice bath, and methanesulfonyl chloride (1.5 μL, 2.2 mg, 0.0190 mmol) was added. The ice bath was removed, and, after one h, 4.0 mL of water were added. This solution was extracted with ethyl acetate (3 × 4 mL). The combined organic extracts were washed with 2*M* aqueous hydrochloric acid (2 × 4 mL) and brine (2 × 4 mL). After drying over anhydrous magnesium sulfate the solvent was removed *in vacuo*. The residue was purified by short path column chromatography (2 g of silica gel, methylene chloride/ethyl acetate, 10:1) to afford a yellow solid (5.8 mg, 87%, mp 223–224 °C dec.). IR ($CHCl_3$): 2935 (br), 2840, 1640, 1595, 1400, 1340, 1330, 1300, 1270, 1180, 1165, 985, 970, 890, 855 cm^{-1}; ^1H NMR ($CDCl_3$): 7.48 (d, *J*= 2.4 Hz, 1H, H-5), 7.23 (s, 1H, H-4), 6.81 (d, *J* = 2.4 Hz, 1H, H-7), 5.53 (d, *J* = 4.9 Hz, 1H, H-1'), 5.21 (br d x t, *J* = *ca.* 5, 10 Hz, 1H, H-2'), 4.04 (s, 3H, OMe), 4.00 (s, 3H, OMe), 3.13 (s, 3H, SO_2Me), 1.9–2.3 (3 x cm, 4H, H-3', -4'), 1.60 (s, 3H, H-6'); MS, *m/z* 490 (M^+, 18%), 411, 394, 351 (100%), 337, 325, 313, 91; accurate mass 490.0933, calcd for $C_{23}H_{22}O_{10}S$ 490.0933.

2'-(*O*Methanesulfonyl)-6,8-di-*O*-methylnidurufin (15, R'=H). A 25 mL round-bottomed flask was charged with 6,8-di-*O*-methylnidurufin (17.2 mg, 0.0417 mmol), 6.25 mL of methylene chloride and triethylamine (19.5 μL, 14.2 mg, 0.140 mmol). The reaction mixture was cooled to 0 °C with an ice bath and methanesulfonyl chloride (6.5 μL, 9.6 mg, 0.0834 mmol) was added. The reaction took place over 25 min. with stirring , at which time it was quenched by the addition of 7 mL of water. The mixture was extracted with ethyl acetate (3 × 6 mL). The combined organic extracts were washed with 2*M* aqueous hydrochloric acid (2 × 6 mL), brine (2 × 6 mL) and dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification was by short path column chromatography (3 g of silica gel, methylene chloride:ethyl acetate, 12:1) to afford pure mesylate **15** (R'=Ms; 15.1 mg, 74%; mp 211–211.5 °C dec., recrystallized from methylene chloride/ethyl acetate). IR (KBr): 3500-3340, 2940, 1620, 1590, 1390, 1325, 1290, 1265, 1245, 1215, 1165, 980, 940, 840, 755 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.67 (s, 1H, OH), 7.46 (d, *J* = 2.5 Hz, 1H, H-5), 7.23 (s, 1H, H-4), 6.79 (d, *J* = 2.5 Hz, 1H, H-7), 5.42 (br s, 1H, H-1'), 4.89 (d, *J* = 2.5 Hz, 1H, H-2'), 4.03 (s, 3H, OMe), 3.98 (s, 3H, OMe), 3.18 (s, 3H, SO_2Me), 1.9–2.3 (cm, 4H, H-3', -4'), 1.65 (s, 3H, H-6'); MS, *m/z* 490 (M^+, 41%), 412, 394, 366, 352 (100%), 337, 323, 307, 293, 279; FAB accurate mass 491.1014, calcd for $C_{23}H_{23}O_{10}S$ 491.1012.

***exo*-Trifluoroethyl Ether 15 (R'=CH_2CF_3) and *endo*-Trifluoroethyl Ether 16 (R'=CH_2CF_3).** A 5 mL round-bottomed flask was charged with mesylate **15** (R'=Ms; 4.8 mg, 0.0098 mmole) and 6.0 mL of a 2% v/v solution of triethylamine in trifluoroethanol. The reaction was stirred at 80 °C for 2 h, at which time TLC analysis showed the complete disappearance of starting material and the formation of two high R_f materials. The solvent was removed *in vacuo*, and the relative amounts of the two products was determined by HPLC analysis on a Partisil 10 Polyamino Cyano column (Whatman) in 97.5:2.5 chloroform/acetonitrile, in which a flow rate of 1 mL/min. was employed. In this solvent system the less polar material (major product) had a retention time of 5.5 min. and the more polar material (minor product) had a retention time of 7.0 min. By a comparison of the intensity of the absorption of the two products at 290 nm, their ratio was determined to be 8.8:1. Isolation of the two materials was carried out by short path column chromatography (3 g of silica gel, 4:1 pentane/ethyl acetate). The faster running material (R_f = 0.19 in pentane:ethyl acetate, 4:1) was determined to be *exo*-trifluorethyl ether **15** (R'=CH_2CF_3; 3.8 mg, 79%, mp 160–163 °C). IR ($CHCl_3$): 2920, 2850, 1720, 1670, 1620, 1595, 1320, 1135–1300, 1000–1100, 995 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.45 (d, *J* = 2.4 Hz, 1H, H-5) 7.18 (s, 1H, H-4) 6.78 (d, *J* = 2.4 Hz, 1H, H-7), 5.16 (d, *J* = 2.5 Hz, 1H, H-1'), 4.10 (d × q, J_{gem} = 12.6 Hz, J_{HF} = 8.6 Hz, 1H, CH_2CF_3), 4.02 (s, 3H, OMe), 3.98 (s, 3H, OMe), 3.96 (d × q, J_{gem} = 12.6 Hz, J_{HF} = 8.6 Hz, 1H, CH_2CF_3), 3.88 (cm, 1H, H-2'), 2.36-2.38 (cm, 3H, H-3', -4'), 1.96-2.05 (cm, 1H, H-3' or H-4'), 1.57 (s, 3H, H-6'); irradiation at δ 2.38 ppm collapsed signal at δ 3.88 to a doublet, *J* = 2.4 Hz; MS, *m/z* 494 (M^+, 1%), 475, 451, 437, 424, 394, 377, 351 (100%), 325; accurate mass 494.1185, calcd for $C_{24}H_{21}O_8F_3$ 494.1184.

mmol). The reaction mixture was cooled to 0 °C with an ice bath, and methanesulfonyl chloride (1.5 μL, 2.2 mg, 0.0190 mmol) was added. The ice bath was removed, and, after one h, 4.0 mL of water were added. This solution was extracted with ethyl acetate (3 × 4 mL). The combined organic extracts were washed with 2*M* aqueous hydrochloric acid (2 × 4 mL) and brine (2 × 4 mL). After drying over anhydrous magnesium sulfate the solvent was removed *in vacuo*. The residue was purified by short path column chromatography (2 g of silica gel, methylene chloride/ethyl acetate, 10:1) to afford a yellow solid (5.8 mg, 87%, mp 223–224 °C dec.). IR ($CHCl_3$): 2935 (br), 2840, 1640, 1595, 1400, 1340, 1330, 1300, 1270, 1180, 1165, 985, 970, 890, 855 cm^{-1}; ^1H NMR ($CDCl_3$): 7.48 (d, *J*= 2.4 Hz, 1H, H-5), 7.23 (s, 1H, H-4), 6.81 (d, *J* = 2.4 Hz, 1H, H-7), 5.53 (d, *J* = 4.9 Hz, 1H, H-1'), 5.21 (br d x t, *J* = *ca.* 5, 10 Hz, 1H, H-2'), 4.04 (s, 3H, OMe), 4.00 (s, 3H, OMe), 3.13 (s, 3H, SO_2Me), 1.9–2.3 (3 x cm, 4H, H-3', -4'), 1.60 (s, 3H, H-6'); MS, *m/z* 490 (M^+, 18%), 411, 394, 351 (100%), 337, 325, 313, 91; accurate mass 490.0933, calcd for $C_{23}H_{22}O_{10}S$ 490.0933.

2'-(*O*Methanesulfonyl)-6,8-di-*O*-methylnidurufin (15, R'=H). A 25 mL round-bottomed flask was charged with 6,8-di-*O*-methylnidurufin (17.2 mg, 0.0417 mmol), 6.25 mL of methylene chloride and triethylamine (19.5 μL, 14.2 mg, 0.140 mmol). The reaction mixture was cooled to 0 °C with an ice bath and methanesulfonyl chloride (6.5 μL, 9.6 mg, 0.0834 mmol) was added. The reaction took place over 25 min. with stirring , at which time it was quenched by the addition of 7 mL of water. The mixture was extracted with ethyl acetate (3 × 6 mL). The combined organic extracts were washed with 2*M* aqueous hydrochloric acid (2 × 6 mL), brine (2 × 6 mL) and dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification was by short path column chromatography (3 g of silica gel, methylene chloride:ethyl acetate, 12:1) to afford pure mesylate **15** (R'=Ms; 15.1 mg, 74%; mp 211–211.5 °C dec., recrystallized from methylene chloride/ethyl acetate). IR (KBr): 3500-3340, 2940, 1620, 1590, 1390, 1325, 1290, 1265, 1245, 1215, 1165, 980, 940, 840, 755 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.67 (s, 1H, OH), 7.46 (d, *J* = 2.5 Hz, 1H, H-5), 7.23 (s, 1H, H-4), 6.79 (d, *J* = 2.5 Hz, 1H, H-7), 5.42 (br s, 1H, H-1'), 4.89 (d, *J* = 2.5 Hz, 1H, H-2'), 4.03 (s, 3H, OMe), 3.98 (s, 3H, OMe), 3.18 (s, 3H, SO_2Me), 1.9–2.3 (cm, 4H, H-3', -4'), 1.65 (s, 3H, H-6'); MS, *m/z* 490 (M^+, 41%), 412, 394, 366, 352 (100%), 337, 323, 307, 293, 279; FAB accurate mass 491.1014, calcd for $C_{23}H_{23}O_{10}S$ 491.1012.

***exo*-Trifluoroethyl Ether 15 (R'=CH_2CF_3) and *endo*-Trifluoroethyl Ether 16 (R'=CH_2CF_3).** A 5 mL round-bottomed flask was charged with mesylate **15** (R'=Ms; 4.8 mg, 0.0098 mmole) and 6.0 mL of a 2% v/v solution of triethylamine in trifluoroethanol. The reaction was stirred at 80 °C for 2 h, at which time TLC analysis showed the complete disappearance of starting material and the formation of two high R_f materials. The solvent was removed *in vacuo*, and the relative amounts of the two products was determined by HPLC analysis on a Partisil 10 Polyamino Cyano column (Whatman) in 97.5:2.5 chloroform/acetonitrile, in which a flow rate of 1 mL/min. was employed. In this solvent system the less polar material (major product) had a retention time of 5.5 min. and the more polar material (minor product) had a retention time of 7.0 min. By a comparison of the intensity of the absorption of the two products at 290 nm, their ratio was determined to be 8.8:1. Isolation of the two materials was carried out by short path column chromatography (3 g of silica gel, 4:1 pentane/ethyl acetate). The faster running material (R_f = 0.19 in pentane:ethyl acetate, 4:1) was determined to be *exo*-trifluorethyl ether **15** (R'=CH_2CF_3; 3.8 mg, 79%, mp 160–163 °C). IR ($CHCl_3$): 2920, 2850, 1720, 1670, 1620, 1595, 1320, 1135–1300, 1000–1100, 995 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.45 (d, *J* = 2.4 Hz, 1H, H-5) 7.18 (s, 1H, H-4) 6.78 (d, *J* = 2.4 Hz, 1H, H-7), 5.16 (d, *J* = 2.5 Hz, 1H, H-1'), 4.10 (d × q, J_{gem} = 12.6 Hz, J_{HF} = 8.6 Hz, 1H, CH_2CF_3), 4.02 (s, 3H, OMe), 3.98 (s, 3H, OMe), 3.96 (d × q, J_{gem} = 12.6 Hz, J_{HF} = 8.6 Hz, 1H, CH_2CF_3), 3.88 (cm, 1H, H-2'), 2.36-2.38 (cm, 3H, H-3', -4'), 1.96-2.05 (cm, 1H, H-3' or H-4'), 1.57 (s, 3H, H-6'); irradiation at δ 2.38 ppm collapsed signal at δ 3.88 to a doublet, *J* = 2.4 Hz; MS, *m/z* 494 (M^+, 1%), 475, 451, 437, 424, 394, 377, 351 (100%), 325; accurate mass 494.1185, calcd for $C_{24}H_{21}O_8F_3$ 494.1184.

The more polar product (R_f = 0.10 in pentane:ethyl acetate, 4:1) was determined to be the *endo*-tri-fluoroethyl ether **16** (R'=CH_2CF_3; 0.4 mg, 9%, mp 194–198 °C). IR ($CHCl_3$): 2925, 2850, 1620, 1595, 1325 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.45 (d, J = 2.8 Hz, 1H, H-5), 7.17 (s, 1H, H-4), 6.78 (d, J = 2.8 Hz, 1H, H-7), 5.25 (d, J = 4.7 Hz, 1H, H-1'), ABX_3 system in which the center AB four lines appear at δ 4.05 and 4.02 (J_{HF} = 8.9 Hz, 2H, CH_2CF_3) and in which the outer AB four lines are not discernable, 4.00 (s, 3H, OMe), 3.96 (s, 3H, OMe), 3.93 (m, 1H, H-2'), 1.92–2.30 (br m, 4H, H-3', -4'), 1.60 (s, 3H, H-6'); irradiation at δ 2.10 simplifies the multiplet centered at δ 3.93 considerably; MS, *m/z* 494 (M^+, 2%) 451, 424, 394, 351 (100%), 325; accurate mass 494.1178, calcd for $C_{24}H_{21}O_8F_3$ 494.1188.

Mixed Acetal 18. A 10 mL round-bottomed flask was charged with mesylate **15** (R'=Ms; 10.0 mg, 0.0204 mmol) and 5 mL of trifluoroethanol. The reaction was brought to reflux and allowed to stir for 19 h. The solvent was then removed *in vacuo* and the residue was purified by short path column chromatography (3 g of silica gel, pentane:ethyl acetate, 2:1). Isolated was 5.3 mg of a mixture of mixed acetal **18** and furanoanthraquinone **17**. The relative amounts of each product were estimated at 4:1 by integrating their respective resonances in the ^1H NMR spectrum. The yield of mixed acetal **18**, which could be isolated after fractional crystallization from ether/pentane (mp 158–160 °C), was 44%. IR ($CHCl_3$): 2930, 1710, 1630, 1600, 1385, 1350, 1330, 1295, 1165 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.51 (s, 1H, OH), 7.46 (d, J = 2.2 Hz, 1H, H-5), 7.29 (s, 1H, H-4), 6.79 (d, J = 2.2 Hz, 1H, H-7), 5.55 (d, J = 1.2 Hz, 1H, H-1'), ABX_3 system in which the AB four lines are centered at δ 4.13, 4.10, 4.06 and 4.04 (J_{HF} = 8.6 Hz, J_{AB} = 12.0 Hz, 2H, CH_2CF_3), 4.03 (s, 3H, OMe), 3.99 (s, 3H, OMe), 3.54 (br t, J = 6.9 Hz, 1H, H-2'), 2.61 (t, J = 7.3 Hz, 2H, H-4'), 2.17 (s, 3H, H-6'), 2.05 (q, J = 7.1 Hz, 2H, H-3'); MS, *m/z* in EI no M^+ was observed, 475, 451, 437, 423, 394, 377, 351 (100%), 337, 325; FAB accurate mass 495.1267, calcd for $C_{24}H_{21}O_8F_3$ 495.1262. The yield of benzofuran **17**, which could not be isolated in pure form, was 11%. ^1H NMR ($CDCl_3$): diagnostic resonances were observed at δ 7.89 (s, 1H, H-1'), 7.52 (d, J = 2.4 Hz, 1H, H-5), signals for H-4 and H-7 were obscured by those of **18**, 4.05 (s, 3H, OMe), 4.00 (s, 3H, OMe), 3.12 (t, J = 7.4 Hz, 2H, H-3'), 2.93 (t, J = 7.4 Hz, 2H, H-4'), 2.17 (s, 3H, H-6'). For characterization of the 6,8-dihydroxy compound corresponding to **17** see ref. 3.

Kinetics. All rearrangement kinetics were performed with a Partisil 10 Polyamino Cyano analytical column. The concentrations of the starting materials were monitored by measuring the areas of the absorbance peaks at 290 nm. The exact amount of mesylate **15** (R'=Ms) used was not directly measured due to the small amounts of material required for the kinetics analysis. To a 5 mL round-bottomed flask was transferred the volume of a solution of mesylate **15** (R'=Ms) in chloroform necessary to give, after having been diluted to a total volume of 3 mL, an absorbance reading of about three quarters scale on the recorder trace at an absorbance attenuation setting of 16. To serve as an internal standard, 300 μL of a 4.7×10^{-3} *M* solution of anthraquinone in chloroform were also added to the round-bottomed flask. The solvent was removed *in vacuo*. The round-bottom flask was then purged with nitrogen, fitted with a reflux condenser, a gas inlet was connected to a nitrogen source and mineral oil bubbler, and 3.0 mL of trifluoroethanol were added. The reaction vessel was immersed in an 80 °C oil bath and allowed to stir. Periodically, 80 μL were removed from the reaction mixture, the solvent removed under reduced pressure, and the residue was subjected to high vacuum for 10 min. Chloroform (160 μL) was added to dissolve the residue from which 20 μL aliquots were withdrawn for the HPLC analysis using a 10 μL loop injector. For a flow rate of 1 mL/min. and a chart speed of 1 cm/min. a table of retention times in the respective solvent systems used is presented below. The disappearance of starting mesylate was monitored relative to the internal anthraquinone standard and gave excellent first-order plots over greater than four half-lives when fitted by a standard least-squares analysis.

Table I. Retention times of compounds subjected to HPLC kinetic analysis on a Partisil 10 Polyamino Cyano analytical column in their respective solvent systems.

Compound	Solvent System	Retention Time
exo-mesylate **15** (R'=Ms)	96/4 $CHCl_3/CH_3CN$	9.0 min
endo-mesylate **16** (R'=Ms)	96/4 $CHCl_3/CH_3CN$	8.5 min
exo-TFE ether **15** (R'=CH_2CF_3)	97.5/2.5 $CHCl_3/CH_3CN$	5.5 min
endo-TFE ether **16** (R'=CH_2CF_3)	97.5/2.5 $CHCl_3/CH_3CN$	7.0 min
anthraquinone	96/4 $CHCl_3/CH_3CN$	3.0 min
mixed acetal **18**	96/4 $CHCl_3/CH_3CN$	7.6 min
benzofuran **17**	96/4 $CHCl_3/CH_3CN$	8.0 min

***exo*-2'-Iodo-6,8-di-*O*-methylaverufin (20, X=I).** To a well stirred solution of 1,3-dihydroxy-6,8-dimethoxy-2-(5-oxo-1-hexenyl) anthraquinone (**19**; 20 mg, 0.050 mmol) and THF (3 mL) was added H_2O (0.5 μL in 0.3 mL of THF), silver oxide (6.6 mg, 0.028 mmol) and iodine (14.0 mg, 0.055 mmol). The mixture was allowed to stir for 2 h at room temperature at which time it was filtered and the precipitate was washed with ethyl acetate (20 mL). The organic layer was washed with brine (2 x 10 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was subjected to flash chromatography (5 g of silica gel) eluting with chloroform to give *exo*-2'-iodo-6,8-di-*O*-methylaverufin (20 mg, 75.9%, mp 192–193 °C dec., recrystallized from chloroform). IR (KBr): 3380 (br), 2900, 1700, 1610, 1585, 1470, 1430, 1380, 1310, 1280, 1140, 970, 870, 830 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.63 (s, 1H, 1-OH), 7.45 (d, J = 2.4 Hz, 1H, H-5), 7.22 (s, 1H, H-4), 6.79 (d, J = 2.4 Hz, 1H, H-7), 5.32 (d, J = 1.6 Hz, 1H, H-1'), 4.62 (br s, 1H, H-2'), 4.03 (s, 3H, OMe), 3.98 (s, 3H, OMe), 2.37 (d x t, J = 9.2, 13.6 Hz, 1H, H-4'$_{ax}$), 1.88-2.01 (cm, 3H, H-3', H-4'$_{eq}$), 1.67 (s, 3H, H-6'); MS, *m/z* 522 (M^+, 10.8%), 395, 337 (100%); accurate mass 522.0163, calcd for $C_{22}H_{19}O_7I$ 522.0176.

***exo*-2'-Bromo-6,8-di-*O*-methylaverufin (20, X=Br).** To a solution of 1,3-dihydroxy-6,8-dimethoxy-2-(5-oxo-1-hexenyl)anthraquinone (**19**; 20 mg, 0.050 mmol), THF (3 mL) and H_2O (1 μL) was added N-bromosuccinimide (9.9 mg, 0.055 mmol). The mixture was stirred for 1 h at room temperature, filtered and the precipitate was washed with ethyl acetate (20 mL). The organic layers were washed with brine (2 x 10 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was subjected to flash chromatography (8 g of silica gel) eluting with chloroform to give *exo*-2'-bromo-6,8-di-*O*-methylaverufin (10.9 mg, 45.4%, mp 260–261 °C dec. recrystallized from chloroform). IR (KBr): 3400 (br), 2920, 1710, 1615, 1590, 1555, 1380, 1290, 1210, 1160, 990, 840 cm^{-1}; ^1H NMR ($CDCl_3$): δ 13.62 (s, 1H, 1-OH), 7.45 (d, J = 2.5 Hz, 1H, H-5), 7.22 (s, 1H, H-4), 6.79 (d, J = 2.5 Hz, 1H, H-7), 5.37 (br s, 1H, H-1'), 4.48 (br s, 1H, H-2'), 4.03 (s, 3H, OMe), 3.98 (s, 3H, OMe), 2.38 (d x t, J = 5.6, 14.0 Hz, 1H, H-4'$_{ax}$), 2.12 (d x d x t, J = 3.6, 5.2, 13.6 Hz, 1H, H-3'$_{ax}$), 1.96 (apparent d x d, J = *ca.* 4, 12 Hz, 2H, H-3'$_{eq}$, H-4'$_{eq}$), 1.60 (s, 3H, H-6'); MS, *m/z* 476 (6.5%), 474 (M^+, 7.7%), 395, 337 (100%); accurate mass 474.0311, calcd for $C_{22}H_{19}O_7Br$ 474.0314.

6,8-Di-*O*-methyl-1'-hydroxyversicolorone (21). *exo*-2'-Iodo-6,8-di-*O*-methylaverufin (**20**, X=I; 5.7 mg, 0.0109 mmol) and silver trifluoroacetate (2.8 mg, 0.0126 mmol) were stirred at *ca.* 4 °C in 1,4-dioxane for 2 h and then at room temperature an additional 18 h. Thin layer chromatography (silica gel, benzene:ethyl acetate, 3:1) indicated some starting material remaining. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel using chloroform as eluent to afford 6,8-di-*O*-methyl-1'-hydroxyversicolorone (**21**; 3.2 mg, 71%, mp 199-202 °C recrystallized from ethyl acetate). IR ($CHCl_3$): 2585, 3355, 3010, 2930, 2850, 1715, 1625, 1600, 1560, 1325, 1290, 1160, 990 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.42 (d, J = 2.4 Hz, 1H, H-5), 7.18 (s, 1H, H-4), 6.78 (d, J = 2.4 Hz, 1H, H-7), 5.80 (d x d, J = 1.2, 6.4 Hz, 1H, H-1'), 4.05 (s, 3H, OMe), 4.02 (s, 3H, OMe), 3.80 (d, J

= 6.4 Hz, 1H, 1'-OH), 3.43 (d x t, J = 1.2, 7.0 Hz, 1H, H-2'), 2.53-2.68 (sym m, 2H, H-4'), 2.16 (s, 3H, H-6'), 1.97-2.06 (sym m, 2H, H-3'); MS, *m/z* 412 (M^+, 3%), 394, 351 (100%), 327, 325; accurate mass 412.1162, calcd for $C_{22}H_{20}O_8$ 412.1158.

Compound 23 with [1-^{13}C]acetate. A mixture of *exo*-2'-iodo-6,8-di-*O*-methylaverufin (**20**, X=I; 24.0 mg, 0.046 mmol), silver [1-^{13}C]acetate (8.6 mg, 0.051 mmol), THF (11.3 mL) and water (1μL) was stirred at room temperature under an argon atmosphere for 2.5 h. The reaction mixture was filtered and the filtrate was diluted with 30 mL of ethyl acetate, washed with dilute aqueous sodium bicarbonate, water, then brine and the organic layer was dried over anhydrous magnesium sulfate. After removal of the solvent under reduced pressure, the products of the reaction were isolated by chromatography on silica gel eluting with chloroform to afford a small amount of 6,8-di-*O*-methylversicolorone (**21**, 1.2 mg, 6.3%) and the [1-^{13}C]acetate trapping product **23** (18.1 mg, 86.7%, mp 213-215 °C recrystallized from toluene). IR ($CHCl_3$): 3540, 3020, 2940, 2840, 1750, 1670, 1615, 1600, 1390, 1325, 1250, 1230, 1220, 1205, 1140, 990, 860 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.45 (d, J = 2.4 Hz, 1H, H-5), 7.17 (s, 1H, H-4), 6.78 (d, J = 2.4 Hz, 1H, H-7), 6.38 (d x t, J = 0.8, 2.8 Hz, 1H, H-1'), 4.02 (s, 3H, OMe), 3.98 (s, 3H, OMe), 3.81-3.84 (sym m, 1H, H-2'), 2.34-2.46 (cm, 3H, H-3', -4'), 2.14 (s, 3H, COMe), 2.02-2.12 (cm, 1H, H-3'), 1.59 (s, 3H, H-6'); irradiation of the multiplet at δ 2.1 collapsed the signal at δ 6.38 to a clean triplet (J = 2.8 Hz) and altered the multiplets at δ 3.8 and 2.4; irradiation at δ 3.8 simplified the signal at δ 6.38 (d x d, J = 0.8, 2.8 Hz) and altered the resonances at δ 2.4 and 2.1; irradiation of the large multiplet at δ 2.4 greatly simplified those at δ 2.1 and 3.8, but had no effect on that at δ 6.38; MS (unlabeled), m/z 454 (M^+, 4.2%), 414, 394, 351 (100%), 325, 314, 271; accurate mass 454.1276, calcd for $C_{24}H_{22}O_9$ 454.1264.

Acknowledgement. We are pleased to thank S. B. Christensen for the early development of a synthetic route to **13**, S. W. Brobst and T. L. Graybill for obtaining spectral data on several compounds, and Dr. J. L. Kachinski for providing mass spectral analyses in the Department of Chemistry. The National Institutes of Health are gratefully acknowledged for financial support of this research (ES 01670) and, in conjunction with the National Science Foundation, for major funding to acquire the analytical instrumentation used (NMR: RR 01934 and PCM 83-03176; MS: RR 02318; FTIR: BRSG grant).

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