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CD Additivity in Trichothecene Benzoates: Application as a Microanalytical Method for Trichothecene Characterization

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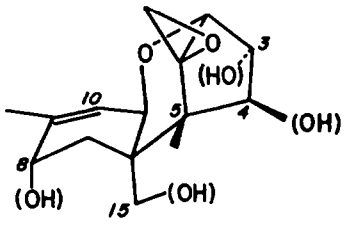
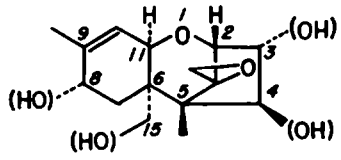
ABSTRACT: The additivity relation in A values of split CD curves previously established with pyranose p-bromobenzoates was shown to be valid for p-bromobenzoates of the cage-like trichothecene system (which also possesses a primary hydroxyl group). This suggests that additivity is generally valid even for congested multichromophoric molecules. The relation has been integrated into a new micro method for trichothecene characterization, which was applied to a new trichothecene from Fusarium sporotrichioides (MC-72083) at a sub- μg level.

When two or more chromophores are located nearby in a chiral molecule or environment, the through-space interactions of the chromophoric electric transition moments give rise to bisignate CD curves. The signs of these so-called split CD curves, governed by the coupled oscillator theory[1] or group polarizability theory,[2] are determined by the chirality of interaction. The signs and amplitudes (defined as the difference in extrema between the two Cotton effects, A values) of the bisignate CD curves have therefore been applied to numerous systems to determine absolute configurations, conformations, etc. ("exciton chirality method").[3]

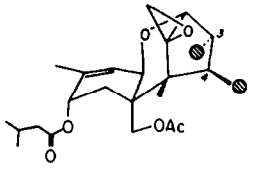
From previous work with hexopyranose p-bromobenzoates[4] it was shown that the A values of split CD curves of tri- and tetrabenzoates can be approximated by the sum of constituent dibenzoate A values, which are constants. This additivity relation was found to be true for benzoate and enone interactions in complex ecdysteroid enone benzoates as well.[5] We have recently found that the additivity is also valid for the flexible p-substituted benzyl ethers of hexopyranoses,[6,7] and are placing considerable effort into developing an additivity-based method for determining glycosidic linkages in oligosaccharides and saponins which does not rely on comparisons with reference sugar derivatives. Delineation of the extent of this additivity rule is thus important from both practical and theoretical viewpoints. If it is established from further examples that the additivity is experimentally and theoretically valid in general, then the interpretation of CD curves would clearly be greatly simplified.

The trichothecenes provide an interesting case for testing the additivity relation because unlike the sugar and steroid systems, they are cage compounds possessing up to four hydroxyls (including primary and allylic hydroxyls)[8] in a congested space.

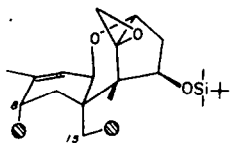




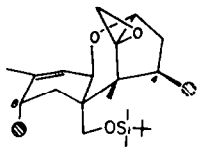
Furthermore, they are a growing class of important toxic sesquiterpenoids[9] produced by a variety of fungi such as Fusarium, Stachybotrys, Cephalosporium, Myrthecium and Trichothecium. They are directly responsible for causing significant diseases in humans and agricultural animals exemplified by alimentary toxic aleuka, skin inflammation, vomiting, anorexia, weight loss and death.[10] If the additivity relation holds for the trichothecenes, it would then be possible to determine the number and positions of free hydroxyls in unknown samples on a submicrogram level. As described in the following, these objectives have been achieved.



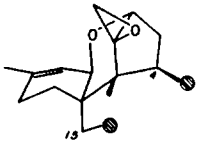
1
3,4-: 253(+39)/236(-13)
A +52



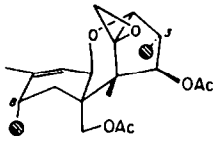
2
8,15-: 254(-59)
A -59



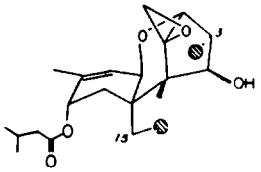
3
4,8-: 250(-42)
A -42



4
4,15-: 255(+2)/236(-9)
A +11



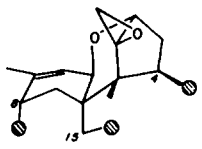
5
3,8-: (+25)/236(-18)
A +43



6
3,15-: 251(+18)
A +18

Fig. 1. CD data of di-p-bromobenzoates, in MeCN. Extrema in nm, $\Delta\varepsilon$ values in ( ), and A values are given. Hatched circles denote p-bromobenzoate group.

In order to determine the standard values for dibenzoate interactions in this system, the six possible permutations of di-p-bromobenzoates were prepared from the corresponding natural diol or from verrucarol (Fig. 1, 4 with 4,15 diol) by various synthetic routes. These values were subsequently used in calculating A values for tri- and tetrabenzoates 7, 8, and 9 (Fig. 2).

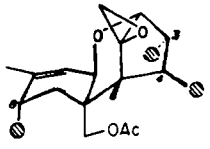


7

4,8,15-: 253(-90)

A -90

4,8	-42
4,15	+11
8,15	-59
	-90

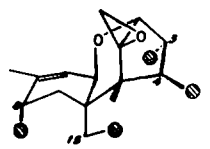


8

3,4,8-: 254(+33)/237(-24)

A +57

3,4	+52
3,8	+43
4,8	-42
	+53



9

3,4,8,15-: 251(+11)/234(-14)

A +25

3,4	+52
3,8	+43
3,15	+18
4,8	-42
4,15	+11
8,15	-59
	+23

Fig. 2. CD data of tri- and tetra- p-bromobenzoates. Experimental A values are compared with A values estimated from sums of dibenzoate contributions.

Since the amount of benzoates used in these studies was 1 mg or less, the concentrations of solutions were calculated from the UV absorbance at 245 nm.[11] The excellent agreement between the calculated and observed A values shows that the additivity relationship does hold for benzoates of the congested trichothecene system; the agreement is quite remarkable considering the fact that the calculated value for tetrabenzoate 9 is derived from a total of six dibenzoate interactions, including the primary hydroxyl derivative.

What these numerical results do not show, however, is that the additivity relationship holds despite the fact that some of the CD spectra showed interference from Cotton effects not derived from the L_a transition of the p-bromobenzoate chromophore (Fig. 3). For example, dibenzoate 2 showed only a single Cotton effect rather than the customary biphasic split CD. However, it is to be noted that the position of the Cotton effect is at 254 nm which is the expected wavelength for interacting p-bromo-benzoate chromophores; if the two benzoates were not interacting, the Cotton effect would have been centered at the λ_{max} of the chromophore, i.e., 245 nm. The atypical shape of the CD curve may be attributable to the interaction of the 8-OBz group with the 9-ene. A precedence of such a non-split CD curve resulting from the two interacting benzoate groups has been

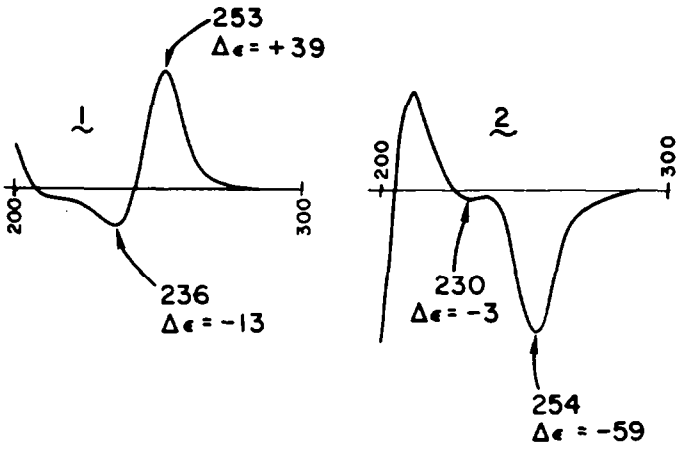


Fig. 3. CD spectra of 1 and 2, in MeCN.

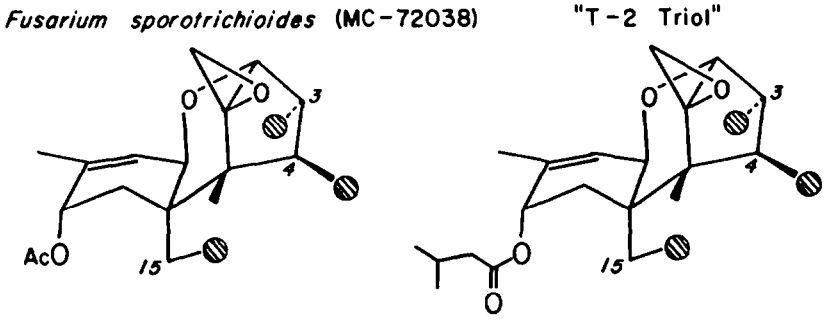


Fig. 4. CD and MS data of two trichothecene tri-p-bromobenzoates.

encountered in cholest-5-ene-3,4-dibenzoate,[12] whose CD displayed a single negative Cotton effect at the perturbed wavelength of 236 nm, $\Delta\epsilon$=-19.0; this sample also contains an allylic benzoate system.[8] Moreover, in many of the sugar dibenzoates measured to date, quite often the CD curves do not appear to be typically bisignate, the longer wavelength Cotton effect being far stronger than that of the shorter.

The additivity relation was next applied on a micro-scale (Fig. 4) to the new trichothecene 8-acetyl T-2 tetraol (10: 3,4,15-triol) isolated from the fungus Fusarium sporotrichioides (MC-72083).[13] Although the structure was characterized by NMR and other data, it will be described here as a hypothetical unknown trichothecene derivative; however the method should be adaptable to real cases. The MS of the bromobenzoate (Fig. 1), showed a peak at m/z 891(M+1), which together with its characteristic cluster of isotopic peaks showed it to be a tri-p-bromobenzoate; another peak at m/z 830 corresponded to a loss of AcO thus indicating the original sample to be a trichothecene triol acetate. The A value of the tribenzoate (measured with 400 nanograms) was +75. The A values of the four possible tribenzoates estimated from constituent dibenzoates are shown in Fig. 4; only the 3,4,15-tribenzoate is close to the measured value of +75 and hence the compound in question is 8-acetoxy-3,4,15-tri-p-bromobenzoate. A parallel experiment was run with the tribenzoate prepared from the known T-2 triol 11 (Fig. 4). The MS and CD data are in full agreement with the interpretation of those for compound 10.

In view of the additivity relationship encountered in the trichothecene skeleton, it can be stated that additivity is expected to be valid for other congested systems containing multiple benzoate groups (and other chromophores). The dibenzoate A values given in Fig. 1 can be employed to determine the configuration and position of free hydroxyls in newly isolated trichothecene fractions[10] on a micro-scale by benzoylation and submitting HPLC fractions to MS and CD measurements. The method should be particularly useful where the amount is insufficient for NMR characterization.[14]

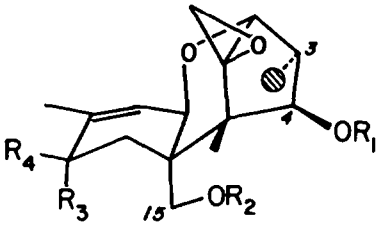
Experimental

All reactions were carried out under an atmosphere of N_2. ^{1}H-NMR spectra were recorded with a Bruker WM-250 (250 MHz) FT spectrometer in deuteriochloroform (unless otherwise specified), with tetramethylsilane as an internal reference. Mass spectra were obtained with a Ribermag-10-10 spectrometer in the DCI mode using NH_3 as the carrier gas. UV spectra were recorded in MeCN with a Perkin Elmer 320 double beam spectrophotometer and were corrected for background absorbance. CD spectra were recorded in MeCN (unless otherwise specified) with a Jasco J-500A spectropolarimeter. Each spectrum was averaged (2 scans) and was corrected for background absorbance (2 scans).

All samples used for UV/CD measurements were purified by HPLC (YMC-SiO_2 5μ, 23% EtOAc/Hex). Concentrations of the solutions used for UV/CD measurements were calculated from their respective A_{245} values using reported ϵ values.[4]

Preparation of 1

Pyridine (0.2ml) was added to a mixture of HT-2 toxin (1:3,4-diol, 7.5mg, 17.7μmol), DMAP("crystal"), and p-bromobenzoylchloride (22.4mg, 102 μmol). The resulting solution was stirred at 65°C overnight. Excess acid chloride was quenched with MeOH(5 drops) and the solution was transferred with excess toluene and evaporated in vacuo. The product was purified by preparative TLC (SiO_2, 25% EtOAc/Hex) to yield 1(6.1mg,58%): R_f= 0.24(25% EtOAc/Hex); MS: 807(M+1+NH_3),730(M-OAc),688(M-OiVal); ^{1}H-NMR: δ8.0-7.91 and 7.65-7.57(2AB's,Ar), 6.34(d,4-H), 5.75(d,10-H), 5.31(d,8-H), 5.30(dd,3-H), 4.47,4.18(AB,15-H's), 4.42(d,11-H), 4.08(d,2-H), 3.16,2.90 (AB,13-H's), 2.11(s,-OAc), 1.77(brs,16-H's); CD: 252(+39)/235(-13), A = +52.



12

Preparation of 2a (12:R_1=R_2=TBDMS, R_3=R_4=H)

DMF(0.5ml) was added to a mixture of verrucarol (12:R_1=R_2=R_3=R_4=H, 22.1mg, 84μmol), TBDMS chloride (133mg, 880μmol), and imidazole (84mg, 1.23mmol). The mixture was allowed to stir overnight at 65°C. DMF was removed from the yellow solution in vacuo. The product was purified by flash chromatography (SiO_2, 5% EtOAc/Hex) to yield 2a(43.8mg,99%): R_f= 0.14 (5% EtOAc/Hex); ^{1}H-NMR: δ5.36(brd,10-H), 4.49(dd,4-H), 3.73(d,2-H), 3.61, 3.38(AB,15-H's), 3.46(brd,11-H), 3.00,2.73(AB, 13-H's), 1.70(brs,16-H's), 1.9-1.8(s's,tBu's), 0.84(s,14-H's), 0.1-0.0(s's,Si-CH_3's).

Preparation of 2b(12: R_1=R_2=TBDMS, R_3=H, R_4=OH)

tert-Butyl hydroperoxide (90% Aldrich, 12μl, 0.21mmol) was added to a suspension of SeO_2(7.1mg, 0.70mmol), in CH_2Cl_2 (0.15ml), and was allowed to stir for 0.5h. 2a(50.2mg, 0.10mmol) in excess CH_2Cl_2 was added to the suspension. The excess CH_2Cl_2 was removed with a stream of N_2 to a final volume of approximately 0.1ml. The suspension was stirred for 3 days at room temperature. Benzene (0.2ml) was added and the solvent was removed with a stream of N_2. The residue was transferred to a test tube with ether (4ml) and washed successively with 10% KOH(aq,4x2ml) and sat. NaCl(1x2ml). The ethereal solution was concentrated to approximately 1ml under a stream of N_2 and 50% AcOH in ether (0.1ml) was added dropwise. The solution was placed in an ice bath and Me_2S(0.8ml) was added slowly with stirring. The resulting solution was stirred 4h at room temperature upon which a snowy white precipitate formed. The mixture was neutralized (20% K_2CO_3), washed successively with H_2O(1x2ml) and sat. NaCl(1x2ml), dried ($MgSO_4$), filtered and evaporated. The product was purified by flash chromatography (SiO_2, 40% EtOAc/Hex) to yield 2b(27mg,54%) and minor amounts (<10%) of recovered 2a and the corresponding enone. 2b displayed the following characteristics: R_f= 0.23(40% EtOAc/Hex); MS: 511(M+1), 493(M-H_2O), 453(M-tert-Bu); ^{1}H-NMR: δ5.44(brd,10-H), 4.40(dd,4-H), 4.11(dd,8-H), 3.77(d,2-H), 3.63,3.33(AB,15-H's), 3.46(d,11-H), 3.00,2.87(AB,13-H's), 1.78(brs,10-H's), 0.90-0.85(s's,t-Bu's), 0.81(s,14-H's), 0.1-0.0(s's,Si-CH_3's).

The β stereochemistry of 2b was confirmed by p-bromobenzoylation (as in 1) to yield 2c(12: R_1= R_2=TBDMS, R_3=H, R_4=p-BrBz; 52%) after purification by preparative TLC (SiO_2; 30% EtOAc/Hex): R_f= 0.66(40% EtOAc/Hex); ^{1}H-NMR: δ7.91,7.59(AB,Ar), 5.69(brdd,8-H), 5.58(brd,10-H), 4.48(dd,4-H),

3.80(d,2-H), 3.73,3.47(AB,15-H's), 3.55(d,11-H), 3.03,2.73(AB,13-H's), 1.69(brs,16-H's), 0.95-0.90(s's,t-Bu), 0.82(s,14-H's), 0.15-0.05 (s's,Si-CH_3's); CD(EtOH): $\Delta\epsilon_{244}$= +10.4 confirms β.

Preparation of 2c(12: R_1=R_2=TBDMS, R_3=p-BrBz, R_4=H)

Ether (0.3ml) was added to a mixture of 2b(4.9mg, 9.6μmol), Φ_3P(3.7mg,14.1μmol) and p-bromobenzoic acid (2.7mg,13.4μmol) followed by addition of DEAD(1.7μl, 10.6μmol). The light yellow solution was stirred overnight at room temperature. The reaction mixture was filtered through a plug of glass wool and the solvent was evaporated with a stream of N_2. The product was purified by preparative TLC (SiO_2, 25% EtOAc/Hex) to yield 2c(5.8mg,87%): R_f= 0.32(25% EtOAc/Hex); ^{1}H-NMR: δ7.84,7.58(AB,Ar), 5.78(d,10-H), 5.50(d,8-H), 5.23(dd,4-H), 4.29(d,11-H), 3.95,3.70(AB, 15-H's), 3.76(d,2-H), 2.99,2.72(AB,13-H's), 1.78(brs,16-H's),0.95-0.8 (s's,t-Bu)$_8$ 0.75(s,14-H's), 0.1-0.0(s's,Si-CH_3's); CD: $\Delta\epsilon_{244}$= -13.7 confirms α.

Preparation of 2d(12: R_1=TBDMS, R_2=H, R_3=OH, R_4=H)

A solution of 2c(9.5mg, 13.7μmol) in THF(60μl) was cooled to 0°C and TBAF(1M in THF,60 μl,60 μmol) was added. The solution was allowed to stir at 0°C(20 min), warmed to room temperature and stirred for 1.5h. The resulting orange solution was transferred to a small test tube with EtOAc(2ml) and washed successively with H_2O(3x0.5ml) and sat. NaCl (1x0.5ml), dried ($MgSO_4$), filtered, and evaporated with a stream of N_2. From the resulting mixture of compounds, 2d was obtained by preparative TLC (SiO_2, 75% EtOAc/Hex) as the major product (2.1 mg,39%) along with a lesser amount of 7a(12: R_1=R_2=H, R_3=p-BrBz, R_4=H, 800μg,13%). 2d displayed the following characteristics: R_f= 0.41(75% EtOAc/Hex); MS: 397(M+1), 414(M+1+NH_3), 379(M-H_2O); ^{1}H-NMR: δ5.54(dd,10-H), 4.37(dd,4-H), 4.10(d,8-H), 3.71,3.42(AB,15-H's), 3.73(d,2-H), 3.40(d,11-H), 2.98,2.75 (AB,13-H's), 1.81(brs,16-H's), 0.88(s,t-Bu), 0.80(s,14-H's), 0.09,0.08 (2s's,Si-CH_3's).

7a displayed the following characteristics: R_f= 0.24(75% EtOAc/Hex); MS: 466(M+1), 483 (M+1+NH_3); ^{1}H-NMR: δ7.84,7.58(AB,Ar), 5.78(brd,10-H), 5.58(brd,8-H), 4.68(dd,4-H), 3.88,3.69(AB,15-H's), 3.80(d,2-H), 3.76(d,11-H), 3.10,2.83(AB,13-H's), 3.0-2.9(-OH's), 1.78(brs,16-H's), 0.93(s,14-H's).

Preparation of 2

2d was p-bromobenzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 40% EtOAc/Hex) to yield 2(1.3mg,84%): R_f= 0.35(40% EtOAc/Hex); MS: 763(M+1), 780(M+1+NH_3); ^{1}H-NMR: δ7.4-7.2(2AB's,Ar), 5.78(d,10-H), 5.35(brd,8-H), 5.44(dd,4-H), 4.38,4.23(AB,15-H's), 3.81(d,2-H), 3.70(d,11-H), 3.08,2.86(AB,13-H's), 1.82(brs,16-H's), 0.89 (s,14-H's), 0.84(2s's,t-Bu), 0.4,0.3(2s's,Si-CH_3's); CD: 254(-59), A= -59.

Preparation of 3a(12: R_1=H, R_2=TBDMS, R_3=p-BrBz, R_4=H)

A mixture of AcOH, THF and H_2O(0.5ml,3:1:1) was added to 2c (4.9mg, 7 mol) and the solution was stirred overnight at room temperature. Most of the solvent was evaporated using a stream of N_2. The remaining aqueous mixture was extracted with EtOAc(2ml). The organic solution was washed successively with sat. $NaHCO_3$(2x1ml) and sat. NaCl(1x1ml), dried (Na_2SO_4), filtered and evaporated. The product was purified by preparative TLC (SiO_2, 30% EtOAc/Hex) to yield 3a as the major UV active product (2.4mg,58%): R_f= 0.11(30% EtOAc/Hex); MS: 581(M+1), 598(M+1+NH_3); ^{1}H-NMR: δ7.84,7.58(AB,Ar), 5.79 (brd,10-H), 5.49(d,8-H), 5.33(dd,4-H), 4.27(d,11-H), 3.98,3.64(AB,15-H's), 3.82(d,2-H), 3.09,2.80(AB,13-H's), 1.77(brs,16-H's), 0.89(2s's,t-Bu), 0.85(s,14-H's), 0.04(2s's,Si-CH_3's).

Preparation of 3

3a(2.1mg, 3.6μmol) was p-bromo benzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 30% EtOAc/Hex) to yield 3(2.2mg,80%): R_f= 0.28 (30% EtOAc/Hex); MS: 763(M+1), 780(M+1+NH_3); ^{1}H-NMR: δ7.90-7.82, 7.60-7.54(2AB's,Ar), 6.58(dd, 4-H), 5.83(brd,10-H), 5.52(d,8-H), 4.42 (d,11-H), 4.03,3.76(AB,15-H's), 3.86(d,2-H), 3.15,2.88(AB,13-H's), 1.80 (brs,16-H's), 0.91(2s's,t-Bu), 0.08,0.04(2s's,Si-CH_3's); CD: 250(-42), A= -42.

Preparation of 4

Verrucarol (4: 4,15-diol, 5.6mg, 21.1μmol) was p-bromobenzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 30% EtOAc/Hex) to obtain 4(9.9mg,74%): R_f= 0.27(30% EtOAc/Hex); MS: 633(M+1), 648(M+1+NH_3); ^{1}H-NMR(acetone-d_6): δ7.90-7.80,7.64-7.57(2AB's,Ar), 5.95(dd,4-H), 5.46(brd,10-H), 4.59,4.29(AB,15-H's), 3.93(d,11-H), 3.81(d,2-H), 3.15,2.94(AB,13-H's), 1.72(brs,16-H's), 0.98(s,14-H's); CD: 255(+2)/236(-9), A= +11.

Preparation of 5

Neosolaniol (5: 3,8-diol, 1.8mg, 4.71μmol) was p-bromobenzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 25% EtOAc/Hex) to yield 5(1.2mg,34%): R_f= 0.17(25% EtOAc/Hex); MS: 766(M+1+NH_3); ^{1}H-NMR(acetone-d_6): δ8.1,7.9,7.8-7.7(2AB's,Ar), 6.07(d,4-H), 5.84 (brd,10-H), 5.50(d,8-H), 5.34(dd,3-H), 4.53,4.11(AB,15-H's), 4.46(d,11-H), 3.97(d,2-H), 3.19,3.03(AB,13-H's), 1.81(brs,16-H's), 0.80(s,14-H's); CD: 252(+25)/236(-18), A= +43.

Preparation of 6

T-2 triol (6: 3,4,15-triol, 1.2mg, 3.1μmol) was partially p-bromobenzoylated (p-bromobenzoylchloride: 1.7mg, 7.7μmol; Pyr:0.05ml) as described for 1. The product was purified by preparative TLC (SiO_2, 45% EtOAc/Hex); ^{1}H-NMR: δ7.93,7.77,7.64,7.53(2AB's,Ar), 5.75(brd,10-H), 5.32(d,8-H), 5.09(dd,3-H), 4.55(dd,4-H), 4.41,4.25(AB,15-H's), 4.01 (d,11-H), 3.96(d,2-H), 3.13, 2.90(AB,13-H's), 3.07(d,-OH), 1.83(brs,16-H's), 0.99(s,14-H's); CD: 251(+18), A= +18.

Preparation of 7

7a(0.8mg, 1.72μmol) was p-bromobenzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 60% EtOAc/Hex) to yield 7(0.5mg,35%): R_f= 0.59(75% EtOAc/Hex); MS: 848(M+1+NH_3); ^{1}H-NMR(benzene-d_6): δ7.78, 7.48,7.41,7.2-6.95(3AB's,Ar), 5.86(dd,4-H), 5.53(brd,10-H), 5.38(brd,8-H), 4.70,4.31(AB,15-H's),3.68(d,2-H), 3.33(d,11-H), 2.63,2.36(AB,13-H's), 1.58(brs,16-H's), 0.93(s,14-H's); CD: 253(-90), A=-90.

Preparation of 8

4-deacetylneosolaniol (8: 3,4,8-triol) was p-bromobenzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 30% EtOAc/Hex) to yield 8(yield undetermined): R_f= 0.32(30% EtOAc/Hex); MS: 891(M+1), 908(M+1+NH_3); ^{1}H-NMR(acetone-d_6): δ8.10,7.97,7.92,7.80-7.67(3AB's,Ar), 6.34(d,4-H), 5.87(brd,10-H), 5.52-5.48(m,3-H,8-H), 4.61,4.16(AB,15-H's), 4.55(d,11-H), 4.06(d,2-H), 3.25,3.09(AB,13-H's), 1.83(brs,16-H's), 0.87(s,14-H's); CD: 254(+33)/237(-24), A= +57.

Preparation of 9

T-2 tetraol (9: 3,4,8,15-tetraol, 2.7mg, 9.06μmol), was p-bromobenzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 30% EtOAc/Hex) to yield 9(3.4mg,34%): R_f= 0.30(30% EtOAc/Hex); MS: 1048(M+1+NH_3); ^{1}H-NMR(acetone-d_6): δ8.13,7.91,7.76-7.68,7.52-7.33 (4AB's,Ar),6.21(d,4-H), 5.92(brd,10-H), 5.58(dd,3-H), 5.46(8-H), 4.84,4.40(AB,15-H's), 4.58(d,11-H), 4.10(d,2-H), 3.29,3.19(AB,13-H's), 1.87(brs,16-H's), 1.02(s,14-H's); CD: 251(+11)/234(-14), A= +25.

Preparation of 10

8-Acetoxy T-2 tetraol (10: 3,4,15-triol, 400μg, 1.1μmol) was p-bromobenzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 30% EtOAc/Hex) to yield 10(yield undetermined): R_f= 0.28 (30% EtOAc/Hex); MS: 891(M+1), 908(M+1+NH_3), 831(M-OAc); ^{1}H-NMR: δ7.95-7.80, 7.62,7.54(3AB's,Ar), 6.08(d,4-H), 5.73(brd,10-H), 5.57(dd,3-H), 5.26 (brd,8-H), 4.74,4.40(AB,15-H's), 4.27(d,11-H), 4.08(d,2-H), 3.17,2.95 (AB,13-H's), 1.73(brs,16-H's), 0.92(s,14-H's); CD: 253(+59)/236(-16), A= +75.

Preparation of 11

T-2 triol (11: 3,4,15-triol, 0.9mg, 2.36μmol) was p-bromobenzoylated (as in 1). The product was purified by preparative TLC (SiO_2, 30% EtOAc/Hex) to yield 11(1.1mg,50%): R_f= 0.40(30% EtOAc/Hex);[2] MS: 949 (M+1+NH_3), 829(M-OiVal); ^{1}H-NMR(acetone-d_6): δ8.09,7.95-7.90,7.70-7.65 (3AB's,Ar), 6.25(d,4-H), 5.78(d,10-H), 5.55(dd,3-H), 5.36(d,8-H), 4.75, 4.42(AB,15-H's), 4.56(brd,11-H), 4.06(d,2-H), 3.25,3.12(AB,13-H's), 1.74(brs,16-H's), 1.04(s,14-H's); CD: 253(+55)/235(-22), A= +77.

8-acetoxy T-2 tetraol(10: 3,4,15-triol)

Details of the culture conditions and isolation procedure will be published elsewhere.[13]

8-acetoxy T-2 tetraol, $C_{17}H_{24}O_7$ (m/z 340.1522, calc 340.1531),oil, shows IR bands at 1726(film, ester) and 3422(OH). The ^{1}H-NMR($CDCl_3$, 300 MHz) displayed the characteristic pattern of the 12,13-epoxide methylene protons, along with signals corresponding to 5,9 and acetate methyls. The remaining protons were assigned using COSY. The downfield shift of the 8-H indicated that the acetate resides at C-8. The presence of the trichothecene skeleton was confirmed by ^{13}C-NMR (d_6-acetone,75 MHz).

Detailed NMR characteristics are as follows- ^{1}H-NMR: δ5.78(brd,10-H), 5.41(d,8-H), 4.44(d,4-H), 4.24(dd,3-H), 4.05(d,11-H), 3.82,3.78(AB,15-H's), 3.72(d,2-H), 3.01,2.77(AB,13-H's), 2.33,1.93(m,7-H's), 2.10(s,-OAc), 1.73(brs,16-H's), 0.87(s,14-H's). ^{13}C-NMR: δ167.2(s,-OAc), 135.5(s,C-9), 126.0(d,C-10), 81.3(d,C-4), 80.9(d,C-3), 79.8(d,C-2), 69.4(d,C-8), 68.0(d,C-11), 65.3(s,C-12), 63.1(t,C-15), 49.3(s,C-5), 46.6(t,C-13), 44.9(s,C-6), 21.2(q,-OAc), 20.3(q,C-16), 7.4(q,C-14).

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